UNIVERSITEIT GENT

Universitair Ziekenhuis Gent

ACUTE KIDNEY INJURY IN THE BIOMARKER AREA RESULTS FROM A COHORT OF PROSPECTIVELY INCLUDED PATIENTS WITH SEPSIS, SEVERE SEPSIS OR SEPTIC SHOCK

Promotor: Prof. Dr. Wim Van Biesen
Co-promotor: Prof. Dr. Annemieke Dhondt
Begeleidingscommissie: Prof Dr. E. Hoste

Thesis submitted to the Faculty of Medicine and Health Sciences in fulfillment of the requirements to obtain the degree of Doctor in Medicine and Health Sciences

2014

Cuando la fe mueve montañas, Francis Alÿs, Peru, 2002

This research was funded by "Klinisch Onderzoekfonds UZ Gent doctoraatsproject" (KOF)

Universitair Ziekenhuis Gent

UNIVERSITEIT GENT

Doctoral thesis:

ACUTE KIDNEY INJURY IN THE BIOMARKER AREA: RESULTS FROM A COHORT OF PROSPECTIVELY INCLUDED PATIENTS WITH SEPSIS, SEVERE SEPSIS OR SEPTIC SHOCK.

Doctorandus: Jill Vanmassenhove

Promotor: Prof Dr. Wim Van Biesen

Co-promotor: Prof Dr. Annemieke Dhondt

Begeleidingscommissie: Prof. Dr. Eric Hoste

LIST OF ABBREVIATIONS

A

$\alpha_1$acidGP	alpha 1 acid glycoprotein
ACCP/SCCM	American College of Chest Physicians/Society of Critical Care Medicine
ADH	Anti Diuretic Hormone
ΔADM	the difference between the serum creatinine value 24 hours after ICU admission and the ICU admission value
ADQI	Acute Dialysis Quality Initiative
AHR	Adjusted Hazard RatioAKI: Acute Kidney Injury
AKIboth	AKI based on both criteria (serum creatinine and urinary output), according to RIFLE
AKIc	AKI based on the serum creatinine criterion according to RIFLE
AKIN	Acute Kidney Injury Network
AKIuo	AKI based the urinary output criterion according to RIFLE
α_1MG	alpha 1 microglobulin
AP	Alcaline Phosphatase
APACHE II score	Acute Physiology and Chronic Health Evaluation II score
ANZICS	Australian New Zealand Intensive Care Society Adult Patient Database
ARF	Acute Renal Failure
AST	Aspartate aminotransferase
AUC ROC	Area Under the Curve of a Receiver Operating Characteristics Curve
AXT	aorta cross clamp time

B

B2MG	Beta2 Microglobulin

C

CASP	Colon Ascendens Stent Peritonitis
CIN	Contrast Induced Nephropathy
CK	creatinine kinetics
CKD	Chronic Kidney Disease
CLP	Caecal Ligation and Punction
CM	Clinical Model
COPD	Chronic Obstructive Pulmonary Disease
CPB	Cardiopulmonary Bypass
CPT	Current Procedural Terminology
CrCl	creatinine clearance
CRP	C Reactive Protein

D

Def	definition
DeltaNGAL	the difference between the urinary NGAL value and the serum NGAL value (ng/ml)
DiffNGAL	the difference in urinary NGAL between time points T4 and T0 and between time points T24 and T4, normalised for urinary creatinine

E

€	Euro
ECMO	extracorporal membrane oxygenation

ED	Emergency Department
(e)GFR	(estimated) Glomerular Filtration Ratio
ELISA	Enzyme Linked Immunosorbent Assay
ERBP	European Renal Best Practice
ESI	Electron Spray Ionisation
ΔEST	The difference between the highest serum creatinine value over the first 24 hours after ICU admission and an estimated baseline value
EQ5D questionnaire	Euro Col 5 Dimensions questionnaire

F

FENa	Fractional Excretion of Sodium
FENGAL	Fractional Excretion of Neutrophil gelatinase-associated Lipocalin
FEUrea	Fractional Excretion of Urea
FSGS	Focal Segmental Glomerular Sclerosis
FTIC	Fourier Transform Ion Cyclotron Resonance

G

GGTP	Gamma Glutamyl Trans Peptidase
GST-α	Glutathione-S-Transferase-alpha
GST-π	Glutathione-S-Transferase-pi

H

HES	Hydroxyethyl starch
HGF	Hepatocyte Growth Factor
ΔHIS	The difference between the highest serum creatinine value over the first 24 hours after ICU admission and a historical baseline value

I

ICAM-1	Intracellular Adhesion Molecule-1
ICD-9	International Classification of Diseases, 9th Revision
ICD-10	International Classification of Diseases, 10th Revision
ICD-9-CM	International Classification of Diseases, 9th Revision, Clinical Modification
ICU	Intensive Care Unit
ICUarr	ICU arrival
IgA	Immunoglobulin A
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-10	Interleukin 10
IL-18	Interleukin 18
Imm	immediately
IQT	Inter Quartile Range
IT	Ion Trap

K

kD	kilodalton
KDIGO	Kidney Disease: Improving Global Outcome
KIM-1	Kidney Injury Molecule-1

L

LDH	Lactate Dehydrogenase
LFABP	Liver Fatty Acid Binding Protein
LOS	Length of Stay
LPS	Lipopolysaccharide

M

M	Mortality
MALDI	Matrix-Assisted Laser Desorption Ionisation
MDRD	Modification of Diet in Renal Disease
Mort	Mortality
MP-ICU-T	marker panel-intensive care unit-training set
MP-ICU-V	marker panel-intensive care unit-validation set
MR	Multiple Reaction
MS	Mass Spectrometry

N

NA	Not Available
NAG	N-Acetyl beta Glucosaminidase
NGAL	Neutrophil gelatinase-associated Lipocalin
NHAMCS	National Hospital Ambulatory Medical Care Survey
NIS	Nationwide Inpatient Sample
NPV	Negative Predictive Value
NRI	Net Reclassification Improvement

O

OR	odds ratio

P

PAH	Para-aminohippurate
PAH ER	Para-amminhippurate Extraction Ratio
PAI-1	Plasminogen Activator Inhibitor-1
PCS	Physical Component Score
PCX	Podocalyxin
P	Plasma
POD	postoperative day
PPV	Positive Predictive Value
pRIFLE	paediatric RIFLE
PTCA	Percutaneous Transluminal Coronary Angioplasty

Q

Q	Quadrupole
QALY	Quality adjusted Life Years
QOL	Qality of Life

R

RAAS	Renin Angiotensin Aldosteron System
RAS	Renin Angiotensin System
RBF	Renal Blood Flow

RBP	Retinol Binding Protein
RIFLE	Risk, Injury, Failure, Loss of Kidney Function, End Stage Renal Disease
RIFLE-R	RIFLE-Risk
RIFLE-I	RIFLE-Injury
RIFLE-F	RIFLE-Failure
RR	Relative Risk
RRT	Renal Replacement Therapy

S

S	Serum
$	US Dollar
sCr	Serum Creatinine
Screa	Serum Creatinine
sCysC	serum cystatin C
SD	standard deviation
SIRS	Systemic Inflammatory Response Syndrome
SR	Selected Reaction
St	stage
sTNFR-I	soluble Tumor Necrosis Factor Receptor-I
sTNFR-II	soluble Tumor Necrosis Factor Receptor-II
Sust	Sustained

T

TAKI	Transient Acute Kidney Injury
TNFα	Tumor Necrosis Factor alpha
TOF	Time of Flight
11k-TXB2	11 Thromboxane B2

U

U	Urine
Ucrea	Urinary creatinine
UO	Urinary Output
US	United States

V

VAS	Visual Analogue Scale
vWF	von Willebrand Factor

W

WRF	Worsening Renal Function

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

1.1 Sepsis

1.1.1 Definition

Before 1992 terms like 'bacteraemia', 'septicaemia' and 'sepsis syndrome' were used interchangeably and 'sepsis syndrome' was considered synonymous with 'severe infection'. However, it is generally the response of the host characterised by a marked inflammatory response, rather than the infectious agent itself, that portends a dismal prognosis.[1] The inflammatory host's response can also occur under non-infectious conditions such as trauma, burns or pancreatitis and has been labelled as SIRS (Systemic Inflammatory Response Syndrome). Sepsis can be considered as SIRS caused by an infectious agent. In 1992, the American College of Chest Physicians and Society of Critical Care Medicine (ACCP/SCCM) organised a consensus conference with the goal to define sepsis and organ failure. They agreed that sepsis should be defined as a systemic response to infection and that this systemic response is manifested by two or more of the following conditions as a result of infection: temperature >38° or < 36°, heart rate > 90 beats/min, respiratory rate > 20 breaths/min or $PaCO_2$ < 32 Torr (<4.3 kPa), white blood cells > 12,000 cells/mm^3 or < 4,000 cells/ mm^3, or >10% immature (band) forms. Sepsis associated with organ dysfunction, hypoperfusion, or hypotension (a systolic blood pressure of < 90 mmHg or a reduction of > 40 mmHg from baseline in the absence of other causes for hypotension) was defined as severe sepsis, and septic shock was defined as sepsis with hypotension despite adequate fluid resuscitation, along with the presence of perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria or an acute alteration in mental status.[2]

This definition has created a lot of controversy because the SIRS criteria are overly sensitive and not specific, meaning that most ICU patients would qualify for them. Also, these criteria are not of utility in diagnosing a cause for the syndrome or in identifying a distinct pattern of host response.[3;4] They are descriptive rather than mechanistic and describe clinical syndromes, rather than specific pathophysiological processes.[5] In 2001, a broadly supported consensus meeting evaluated the 1992 definition and although they decided evidence did not support a change in the original definition, they did expand the list of potential signs and symptoms of sepsis reflecting clinical bedside experience and thus providing tools that should alert clinicians for a potential diagnosis of sepsis (Table 1). [6;7]

Table 1: Diagnostic tools for diagnosing sepsis [6;7]

Infection, documented or suspected and some of the following:
General variables – **Fever (>38.3°C)** – **Hypothermia (core temperature <36°)** – **Heart rate >90/min or more than two SD above the normal value for age** – **Tachypnoe**

- **Altered mental state**
- **Significant edema or positive fluid balance (>20ml/kg over 24h)**
- **Hyperglycemia (plasma glucose >140mg/dl or 7.7 mmol/l in the absence of diabetes)**

Inflammatory variables

- **Leucocytosis (>12000/µl)**
- **Leukopenia (<4000/µl)**
- **Normal WBC count with greater than 10% immature forms**
- **Plasma C-reactive protein more than two SD above the normal value**
- **Plasma pro calcitonine more than two SD above the normal value**

Hemodynamic variables

- **Arterial hypotension (SBP <90mmHg, MAP<70mmHg or a SBP decrease >40mmHg in adults or more than 2 SD below normal for age)**

Organ dysfunction variables

- **Arterial hypoxemia (PaO_2/FIO_2<300)**
- **Acute oliguria (urine output < 0.5 ml/kg/h for at least 2 hours despite adequate fluid resuscitation)**
- **Creatinine increase >0.5mg/dl or 44.2 µmol/l**
- **Coagulation abnormalities (INR>1.5 or aPTT>60sec)**
- **Ileus (absent bowel sounds)**
- **Thrombocytopenia (platelet count <100,000/µl)**
- **Hyperbilirubinemia (>4mg/dl or >70µmol/l)**

Tissue perfusion variables

- **Hyperlactatemia (>1mmol/l)**
- **Decreased capillary refill or mottling**

1.1.2 Epidemiology

About 37% to 75% of admissions in medical and surgical ICU's is related to sepsis.[8-12] In a multicenter study in French ICU's, the yearly incidence rate of severe sepsis was estimated at 0.95 episodes per 1000 inhabitants and the total annual number of episodes in ICU's at 56,540.[13] Compared with previous studies from the same group in 1993, these data suggest a 75% increase in the incidence rate of severe sepsis.[14] The increasing incidence in sepsis and severe sepsis in the last decades has been corroborated by several other groups in the United States and Europe (Table 2). It is likely that multiple factors contribute to this rapid increase in incidence. First, the population is aging with an increasing burden of chronic disease. Second, there is also an increased use of invasive procedures and immunosuppressive drugs, chemotherapy and transplantation. Last, there is an increasing microbial resistance.[15] Although the increase in the incidence of sepsis is universal, there is still a wide variability between and even within countries (Table 2). This is most likely due to differences in documentation of sepsis and hospital coding practices. In large databases the ICD-9 (International Statistical Classification of Disease) codes are used to capture patients with severe sepsis by coupling codes for infection and for organ dysfunction. Differences in

the methodology to use these codes can explain the variability in epidemiological data.[15] The total number of sepsis related deaths is consistently increasing over the various studies and septicemia has become the 11th leading cause of death in the US (Centers for Disease Control/National Health Service). However, the mortality in patients with sepsis or case fatality rate is decreasing (Table 2). The latter is probably explained by improvements in care for patients with sepsis due to greater awareness of the condition, more rapid administration of antibiotics and better organisation of ICU care.[16-21]

1.1.3 Costs and quality of life

In a time where health care resources are being cut down, the discussion on health-economics and whether treatment for sepsis patients is cost-effective, cannot be avoided. The costs associated with sepsis treatment and research are immense, reaching up to 16.7 billion$ yearly in the US.[22] Treating patients with sepsis costs more than treating general ICU patients. Edbrook et al[23] demonstrated that patients with severe sepsis or early septic shock spent prolonged periods of time in the ICU and were significantly more expensive to treat than non sepsis ICU patients. In their study, the cost of treating a sepsis patients varied from twice to eleven times more than a non septic ICU patient. Weycker et al[24] showed that per sepsis patient the cost for the index admission was on average 44.600$. If patients survived to hospital discharge, costs went up to 78.500$ at 1 year and 118.800$ at 5 years, per patient. In a Canadian retrospective study, Letarte et al[25] studied 100 patients with severe sepsis and septic shock and found that the mean cost was 11.474$ per episode per case or 1064$ per day. For survivors after day 28 through year 1, the cost was 27,481$. The total cost came to 36.4 to 72.9 million$ per year but was higher if patients survived after day 28, indicating that not only the sepsis episode itself, but also revalidation and re-integration afterwards, are expensive. In Germany, the costs associated with sepsis related admissions go up from 3.6 to 7.7 billion € yearly.[26]

In the last decade, several initiatives to improve the care for patients with sepsis were taken. In 2004, an international group of experts in the diagnosis and management of infection and sepsis, representing 11 organizations, published the first internationally accepted guidelines that the bedside clinician could use for management of severe sepsis and septic shock.[27] These guidelines were converted into 'sepsis bundles', defined as selected sets of interventions or processes of care, that, when implemented as a group provide a more robust picture of the quality of care provided.[28] Although implementing an integrated sepsis protocol resulted in a mean increase in cost of 8,800$ per patient, largely driven by increased ICU Length of Stay (LOS), life expectancy and quality adjusted life years were higher in the group receiving the protocol vs those who did not receive it.[29] The protocol was associated with an incremental cost of 11,274 per quality adjusted life years gained.[29] Surviving ICU without regaining a meaningful Quality of Life (QOL) is not a desirable goal of the critical care process[30], which makes mortality an insufficient measure of ICU outcome.[31] Even if sepsis patients survive up to hospital discharge, they often present with residual

organ dysfunction which may result in invalidating symptoms such as dyspnoe, fatigue, impaired function status and reduced health related quality of life in comparision with the general population.[32-36] However, it remains controversial whether this is specifically due to the sepsis syndrome rather than being related to any form of critical illness. In the latter case, this persistent 'dysfunction' could also be attributed to age and underlying comorbidity. Granja et al[34] compared the post discharge quality of life in critically ill patients with versus without sepsis, by using the EQ5D (Euro Col 5 Dimensions) questionnaire. Patients in the sepsis group had a higher mortality, higher APACHE II score and longer LOS vs the non-sepsis group. There was no difference in QOL between both groups except for 'anxiety' and 'depression', two conditions that appeared to be less prevalent in the sepsis group.

Several other studies[37-44] demonstrated a decrement in QOL in sepsis survivors. However they mostly did not report on QOL pre sepsis and compared with population norms which might confound the relationship between reduced QOL and sepsis.

Overall, there is an increasing interest in quality of life and the (potential) advantages of post discharge care in sepsis patients to improve health related quality of life, as indicated by several trials currently underway on these topics.[45;46]

Table 2: Main studies on the incidence of sepsis over time.

	Time period	Setting	Sepsis definition	Incidence	Mortality	Costs
Angus et al[22]	1995 compared to 1993-1994	Non-federal hospitals (n=847) in 7 US states	Severe sepsis, documented infection and acute organ dysfunction (ICD-9-CM)	- 192,980/6,621,559; yielding national estimates of 751,000 cases - 3 cases/1000 population - 2.26 cases/100 hospital discharges - 1.5% increase per year	Overall hospital mortality 28.6%=251,000 deaths annually	Average cost per case: 22,100$ annual, total cost:16.7 billion$
Martin et al[47]	1997-2000	Non-federal acute care hospitals including approximately 500 hospitals with equal representation of all geographic regions	Several ICD-9-CM codes for sepsis, bacteremia and fungal infections Organ failure was defined by a combination of ICD-9-CM and CPT codes	- 10,319,418 cases of sepsis/750 million hospitalizations - 82.7/100,000 in 1997 vs 240.4/100,000 in 2000	- Hospital mortality 1979-1984: 27.8% - Hospital mortality 1995-2000: 17.9%	NA
Wang et al[48]	2001-2004	Emergency department NHAMCS	Suspected severe sepsis Combination of ICD-9 ED admission codes, triage vital signs and clinical interventions	2,282,000 cases /331,500,000 admissions =571,000/year or 0.69%	NA	NA

Dombrovskiy et al[49]	1993-2003	US community hospitals from the NIS=20% sample of US non-federal short term hospitals	Sepsis ICD-9-CM codes for septicemia and major organ dysfunction	- Sepsis: 8,403,766 (=2.15% of all hospitalizations) - Severe sepsis:2,857,476 (=0.73% of all hospitalizations and 34% of all hospitalizations for sepsis) - Percentage of severe sepsis: 25.6% in 1993 vs 43.8% in 2003 - Annual increase of 8.3% in hospitalizations for severe sepsis (64.7/100,000 in 1993 vs 134.6/100,000 in 2003)	- Mortality rate severe sepsis from 30.3% in 1993 to 49.7% in 2003 - Case fatality rate from 45% in 1993 to 37.7% in 2003 - Annual increase in age adjusted mortality for severe sepsis:5.6% - Annual decrease in case fatality rate:1.4%	NA
Wilhelms et al[50]	1987-2005	ICD-9 and ICD-10 according to previously published methods by Angus et al[22], Martin et al[47] and Flaatten et al[51]	Swedish hospital discharge Register recording all hospital admissions	- Method 1[22]: n=37,990; incidence increased from 0.10/1000 in 1987 to 0.35/1000 in 2005 - Method 2[47]: n=27,655; incidence increased from 0.26/1000 in 1987 to 0.43/1000 in 2005 - Method 3[51]: n=12,512; incidence increased from 0.03/1000 in 1987 to 0.13/1000 in 2005	Hospital Mortality - Method 1[22]:22.1% - Method 2[47]:22.4% - Method 3[51]:29.2%	NA

Lagu et al[52]	2003-2007	Severe sepsis ICD-9-CM codes according to Dombrovskiy et al[49] and organ dysfunction	US community hospitals from the NIS=20% sample of US non-federal short term hospitals	200/100,000 in 2003 vs 300/100,000 in 2007; annual increase of 17.8% per year	- Overall mortality from 75/100,000 in 2003 to 87/100,000 in 2007 - Case fatality rate from 37% to 29% (2% per year decrease in hospital mortality for patients with severe sepsis)	Total cost 15.4 billion$ per year in 2003 vs 24.3 billion$ per year in 2007
Gaieski et al[15]	2004-2009	Sever sepsis Documented infection including ICD-9 code for sepsis or septic shock and the presence of new organ dysfunction or the ICD-9 code for severe sepsis + comparing four previous methods (Martin et al[47], Wang et al[48], Angus et al[22] and Dombrovskiy et al[49])	NIS database containing hospital stays from 1050 hospitals in 44 states	- Method 1[22]:905/100,000 - Method 2[48]:1,031/100,000 - Method 3[49]:300/100,000 - Method 4[47]:369/100,000 - Annual increase of 13-13.3% depending on the method used	- Mortality from 14.7% in 2004 to 29.9% in 2009, depending on the method - For all methods[22;47-49]: decrease in case fatality rate	NA
Kaukonen et al[21]	2000-2012	Severe Sepsis with and without shock were defined by the presence of ≥2 SIRS criteria within the first 24h after ICU admission and either 1)APACHE III admission diagnosis consistent with sepsis or 2)APACHE admission diagnosis consistent with infection accompanied by organ failure	Data from the ANZICS	n=2,708/35,012 in 2000 and n=12,512/100,286 in 2012 which means an increase from 7.2% in 2000 to 11.1% in 2012.	Mortality in severe sepsis decreased from 35% in 2000 to 18.4% in 2012 with an average annual decrease of 1.3%	NA

1.2 Septic Acute Kidney Injury

1.2.1 AKI and RRT in sepsis and the critically ill

Sepsis contributes to AKI in about 30-50% of all AKI cases.[53;54] In a study by Bagshaw et al, comparing septic vs non septic AKI, sepsis was considered the cause of AKI in 47.5% of patients.[53] Neveu et al corroborated this by demonstrating that ARF had a septic origin in 45.5%.[10] In another study by Bagshaw et al, sepsis contributed to early AKI (= in the first 24h of admission) in 32.4% of cases.[55] Brivet el al found 48% of ARF (Acute Renal Failure) to be associated with sepsis in critically ill patients.[56] In a large study in critically ill patients, Uchino et al found that septic shock was the most common contributing factor to ARF.[57] Septic Acute Kidney Injury (AKI) associates with a greater severity of illness than non- septic AKI, which translates in higher mortality rates.[53;58;59] In a study by Ali et al, sepsis was a precipitating factor in 47% of AKI cases and the incidence of AKI and Acute on Chronic Renal Failure were 18.1 and 33.6 per 100,000 population, respectively.[60] The incidence of ARF in the critically ill (septic and non septic) varies between 3-25% according to the definition used and the population studied.[54;56;57;61] In patients with AKI around 1.78 per 100 person years are treated with RRT vs 0.74 per 100 person years in those without AKI.[62] Around 4-6% of critically ill patients are treated with RRT, depending on the inclusion criteria.[12;57;63] In the general population, the estimated incidence of acute RRT need varies widely[54;64-69], going from 2.2/100,000[65] to 28.6/100,000 population years.[54] This variability in incidence is explained by 1) the geographic location, 2) the availability of ICU beds, 3) location of RRT (either only ICU or both ICU and ward), 4) the epoch in which the incidence is estimated and 5) the severity of illness of the included patients.

1.2.2 Definition of AKI

Reaching an agreement on a universally accepted definition of Acute Kidney Injury (AKI) has turned out to be one of the major challenges in the field of nephrology. Despite the advances in technology over the last decades, AKI is still associated with an unacceptable high morbidity and mortality and the absence of a universal definition is an impediment to progress in this domain. It hampers the comparison of epidemiological data between different studies and it results in widely different outcome data even within one specific setting (e.g. cardiac surgery or critically ill setting).

In the last years, several efforts to uniformize the definition of AKI were published. In 2004, the Acute Dialysis Quality Initiative (ADQI) established the RIFLE criteria (Table 3).[70] RIFLE is an acronym that stands for “Risk”, “Injury”, “Failure”, “Loss of Kidney Function” and “End Stage Renal Disease”. It consists of three severity stages (Risk, Injury and Failure) and two outcome stages and is based on both serum creatinine and urinary output criteria, classifying patients according to whichever criterion delivers the worst RIFLE classification (e.g. a patient with RIFLE “Risk” for serum creatinine and RIFLE “Failure” for urinary output

will have a final RIFLE classification as “Failure”). In 2007, the Acute Kidney Injury Network[71] refined the RIFLE criteria by 1) eliminating the need for a baseline serum creatinine and using the admission value as a reference value, 2) removing the eGFR criteria (since eGFR should only be used in steady state conditions which AKI is not by definition) and adding an absolute increase in serum creatinine of 0.3 mg/dl next to a relative 1.5 fold increase (Table 3). The addition of a 0.3 mg/dl increase in the definition relates to several reports on the association of even small increases of serum creatinine with worse outcome, mainly in the cardiac surgery setting[72-79] and 3) including a 48 hours window for the serum creatinine increase to occur. Several studies have compared the AKIN and RIFLE criteria in different settings, mostly concluding that they were of equal value (Table 4). KDIGO finally assembled both the RIFLE and AKIN criteria in their definition[80] which was followed by position statements by various associations in Europe and the United States.[81-84] Since the publication of the KDIGO criteria for AKI, they have been validated in different clinical settings and compared to the existing classification criteria of RIFLE and AKIN, demonstrating good performance for diagnosing AKI (Table 4). In several studies the criteria themselves are still being applied differently which makes it difficult to compare studies that at first glance appear to use the same definitions, but in fact use (substantially) different interpretations of the same definition (Table 4).

Most of these studies also report on outcome whereas these criteria were originally not intended to predict mortality.

Table 3: Comparison of the RIFLE, AKIN, KDIGO and ERBP criteria for AKI

	RIFLE	AKIN	KDIGO	ERBP
Reference or baseline sCr value	Historical baseline value If not available: estimated value according to ADQI	Admission value	Baseline value, unspecified	Admission value
sCr criterion: relative increases within severity stages	Risk: sCr x 1.5-1.9 Injury: sCr x 2-2.9 Failure: sCr x 3 or if sCr>4mg/dl an acute increase of 0.5 mg/dl	Stage 1: sCr x 1.5-2 Stage 2: sCr x 2-3 Stage 3: sCr x 3 or RRT or if sCr>4mg/dl an acute increase of 0.5 mg/dl	Stage 1: sCr x 1.5-1.9 Stage 2: sCr x 2-2.9 Stage 3: sCr x 3 or RRT or acute rise in sCr>4 mg/dl	Stage 1: sCr x 1.5-1.9 Stage 2: sCr x 2-2.9 Stage 3: sCr x 3 or RRT or acute rise in sCr>4 mg/dl
sCr criterion: absolute increases within severity stages	NA	Stage 1 if 0.3mg/dl increase over 48h	Stage 1 if 0.3mg/dl increase over 48h	Stage 1 if 0.3mg/dl increase over 48h
GFR criterion	Risk: 25% decrease in GFR Injury: 50% decrease in GFR Failure: 75% decrease in GFR	NA	In patients < 18 years: eGFR<35 ml/min/1,73m2=stage 3	NA
UO criterion within severity stages	Risk: UO < 0.5 ml/kg/u during 6 hours Injury: UO < 0.5 ml/kg/u during 12 hours Failure: UO < 0.3 ml/kg/u during 24 hours or anuria during 12 hours	Stage 1: UO < 0.5 ml/kg/u during 6 hours Stage 2: UO < 0.5 ml/kg/u during 12 hours Stage 3: UO < 0.3 ml/kg/u during 24 hours or anuria during 12 hours	Stage 1: UO < 0.5 ml/kg/u during 6-12 hours Stage 2: UO < 0.5 ml/kg/u during ≥12 hours Stage 3: UO < 0.3 ml/kg/u during ≥24 hours or anuria during ≥12 hours	Stage 1: UO < 0.5 ml/kg/u during a 6 hour block Stage 2: UO < 0.5 ml/kg/u during two 6 hour blocks Stage 3: UO < 0.3 ml/kg/u during ≥24 hours or anuria during ≥12 hours
Time span for AKI diagnosis	within 7 days	time windows of 48h within 7 days	0.3mg/dl increase over 48h or sCr x 1.5 within 7 days	0.3mg/dl increase over 48h or sCr x 1.5 within 7 days

	RIFLE	AKIN	KDIGO	ERBP
Outcome stages	-Loss: Persistent ARF. Complete loss of kidney function >4 weeks -End Stage Kidney Disease: RRT need ≥3 months	NA	NA	NA
Extra	AKI should be sustained for at least 24 hours	- Exlude obstruction - Staging should be done after fluid resuscitation to exclude prerenal factors	The cause of AKI should be determined wheneverpossible.	- The cause of AKI should be determined whenever possible. As a minimal work-up, the presence of hypovolaemia, post-renal causes, low cardiac output, use of nephrotoxic agents, acute glomerulonephritis and renal micro-angiopathy as underlying contributors to AKI should be evaluated. - Urinary volume should be expressed using ideal body weight rather than real body weight when calculating urinary output in ml/kg/u - Urinary output can be collected in 6 hour blocks instead of hourly - eGFR should not be used

Alhough all these efforts are a major step forward, there are still some flaws and pitfalls when using these definitions. First, as stated above, these classification criteria are originally based on both serum creatinine and urinary output criteria[70;71;80;81] but the latter are mostly omitted because data on hourly or six hourly urinary output are often lacking, especially in large administrative datasets.[85-87] Second, the requirement of a historical baseline serum creatinine for defining absolute or relative serum creatinine increases in RIFLE, is not always satisfied. Using surrogate baseline values such as the admission value, a nadir value during the actual hospitalization or an estimated value by solving the MDRD equation assuming a GFR of 75 ml/min/1,73m2 as suggested by ADQI[70] is susceptible for misclassifications in both directions (either over- or underestimation of AKI).[88;89] Third, serum creatinine is an imperfect parameter for AKI diagnosis because it is influenced by several non-renal factors such as age, gender, race, muscle mass and in sepsis patients, volume status. Also, it is a functional parameter and it increases slowly after a renal insult so that there is a delay in diagnosis.[90] The latter has made the search for new serum and urinary biomarkers that are only influenced by tubular damage, a top priority in the field of nephrology. The additional value of these biomarkers on top of currently used parameters such as serum creatinine and urinary output remains controversial (see also section 1.3 on biomarkers).

Table 4: Studies comparing RIFLE, AKIN and KDIGO critera

Study	Study design	Setting	Included patients	Comparison	AKI criteria	Baseline sCr	Time span AKI diagnosis	Outcome
Bagshaw et al/2008/ NDT[85]	retrospective	critically ill	n=120,123	RIFLE vs AKIN	sCr and UO but modified UO criteria	estimated according to ADQI	first 24h after ICU admission	**AKI:** - RIFLE:36.1% - AKIN:37.1% **Hospital mortality:** - RIFLE:AUC ROC 0.66 - AKIN:AUC ROC 0.67
Lopes et al/2008/Crit Care[91]	retrospective	critically ill	n=662	RIFLE vs AKIN	sCr and UO	- RIFLE: estimated according to ADQI - AKIN: lowest value within 24h after admission	within ICU hospitalization	**AKI:** - RIFLE:43.8% - AKIN:50.4% **Hospital mortality:** - RIFLE:AUC ROC 0.73 - AKIN:AUC ROC 0.75
Haase et al /2009/ J Thorac Cardiovasc Surg[92]	prospective	cardiac surgery	n=282	RIFLE vs AKIN	sCr and UO	NA	RIFLE: within 7 days postoperatively AKIN: within 48h	**AKI:** - RIFLE:45.8% - AKIN:44.7% **Hospital mortality:** - RIFLE:AUC ROC 0.91 (0.82-0.99) - AKIN:AUC ROC 0.94 (0.81-0.97)
Joannidis et al/2009/ICM[86]	retrospective	critically ill	n=16,784	RIFLE vs AKIN	sCr and UO but modified UO criteria	-RIFLE: estimated according to ADQI -AKIN: ICU admission value	First 48h after admission	**AKI:** - RIFLE:35.5% - AKIN:28.5% **Hospital mortality:** - RIFLE-R:OR 1.38(1.17-1.63) - RIFLE-I:OR 1.9(1.65-2.18) - RIFLE-F:OR 2.99(2.66-3.36) - AKIN st 1:OR 2.07(1.77-2.43) - AKIN st 2:OR 1.93(1.63-2.28) - AKIN st 3:OR 2.99(2.64-3.38)

Study	Study design	Setting	Included patients	Comparison	AKI criteria	Baseline sCr	Time span AKI diagnosis	Outcome
Robert et al/2010/ Cardiac Surgery[93]	retrospective	cardiac surgery	n=25,086	RIFLE vs AKIN	sCr only	the latest sCr value before surgery	during hospitalization	**AKI:** - RIFLE:31% - AKIN:30% **Hospital mortality:** - RIFLE:AUC ROC 0.78 - AKIN:AUC ROC 0.77
Yan et al/2010/ Eur J Cardiothorac Surg[94]	retrospective	post cardio-tomy on ECMO	n=67	RIFLE vs AKIN	sCr and UO	the first value available for every patient hospitalized	First 48h after ECMO	**AKI:** - RIFLE:81% - AKIN:85% **Hospital mortality:** - RIFLE-F:AUC ROC 0.74 - AKIN:AUC ROC 0.80
Chang et al/2010/ Shock[95]	retrospective	critically ill	n=291	RIFLE vs AKIN	sCr and UO	the first value at hospitalization or estimated according to MDRD in n=20	NA	**AKI:** - RIFLE:61% - AKIN:% **Hospital Mortality:** - RIFLE:AUC ROC 0.74 - AKIN:AUC ROC 0.72
Englberger et al/2011/Crit Care[96]	retrospective	cardiac surgery	n=4,836	RIFLE vs AKIN	sCr only	the last value before surgery	first 7 postoperative days	**AKI:** - RIFLE:18.9% - AKIN:26.3% **Hospital mortality:** Increase with an OR of 4.5(3.6-5.6) for every increase in RIFLE class vs 5.3(4.3-6.6) for AKIN
Wang et al/2013/NDT[97]	retrospective	Adult in-patients under-going ≥2 sCr measure-ments	n=19,878	KDIGO vs absolute sCr increase	sCr only	lowest of the 3 first sCr values available	during hospitalization	**AKI:** - KDIGO:23.4% - Delta sCr discriminates the differences between adjacent AKI stages earlier **In-patient Mortality:** NRI between delta sCr and KDIGO for prediction of mortality was 9.7%(6.2-13.2%)

Study	Study design	Setting	Included patients	Comparison	AKI criteria	Baseline sCr	Time span AKI diagnosis	Outcome
Roy et al/2013/ Cardiorenal Med[98]	retrospective	heart failure	n=637	KDIGO vs RIFLE,AKIN,WRF	sCr only	estimated from the admission value (if within the normal range) or from another value within 6 months (whichever was lowest)	- RIFLE: 1-7 days and sustained for at least 24h - AKIN: 48h period during hospitalization - KDIGO: 1.5 increase over 7 days or 0.3mg/dl increase over 48h	**AKI:** - KDIGO:36.7% - RIFLE:25.6% - AKIN:27.9% - WRF:33% **Composite outcome (Heart Failure related readmission, RRT need or death)** ***30 days:** - KDIGO:AUC ROC 0.74 - RIFLE:AUC ROC 0.76 - AKIN:AUC ROC 0.72 - WRF:AUC ROC 0.72 ***1 year:** - KDIGO:AUC ROC 0.67 - RIFLE:AUC ROC 0.64 - AKIN:AUC ROC 0.64 - WRF:AUC ROC 0.65
Rodrigues et al/2013/PLoS One[99]	prospective	post AMI	n=1,050	KDIGO vs RIFLE	sCr only	admission sCr	7 days	**AKI:** - KDIGO:36.6% - RIFLE:14.8% **30 days mortality:** - KDIGO:AHR 3.99(2.59-6.15) - RIFLE:AHR 3.51(2.35-5.25) **1 year mortality:** - KDIGO:AHR 2.43(1.62-3.62) - RIFLE:AHR 1.84(1.12-3.01)
Bastin et al/2013/ J Crit Care[100]	retrospective	cardiac surgery	n=1,881	KDIGO vs AKIN,RIFLE	sCr only	most recent sCr value either pre admission or upon admission	first 7 days after admission	**AKI:** - KDIGO:25.9% - RIFLE:24.9% - AKIN:25.9% **Hospital mortality:** - KDIGO: AUC ROC 0.86 - RIFLE:AUC ROC 0.78 - AKIN:AUC ROC 0.86

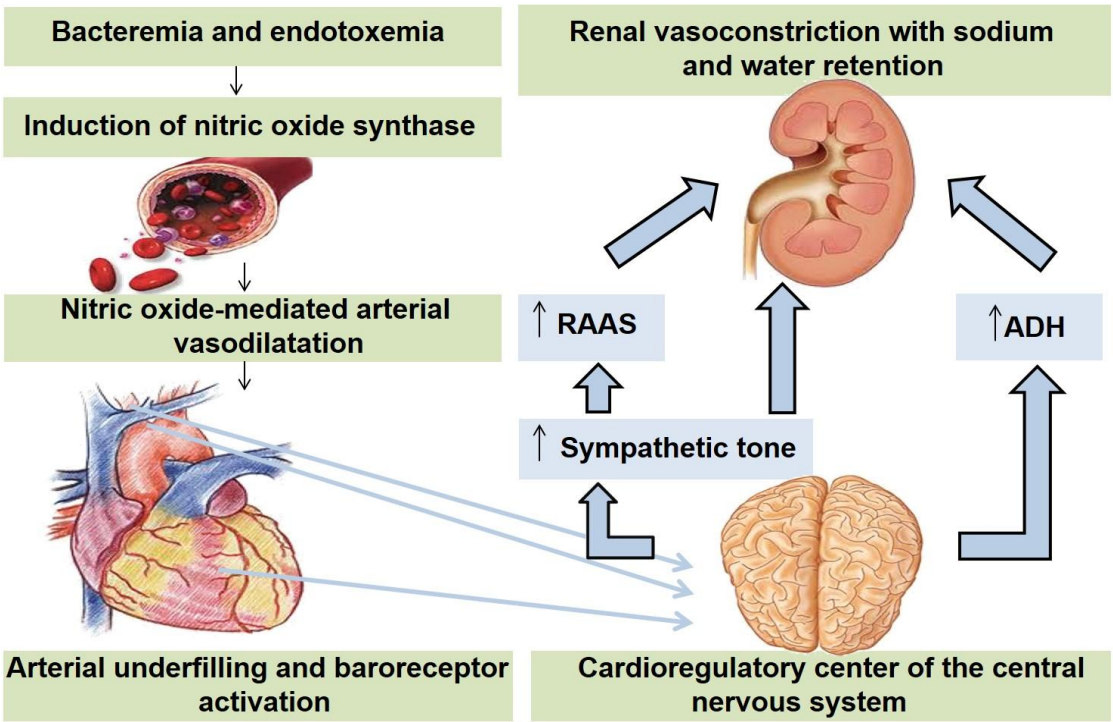
Study	Study design	Setting	Included patients	Comparison	AKI criteria	Baseline sCr	Time span AKI diagnosis	Outcome
Zeng/ 2014/CJASN[101]	retrospective	Hospita-lized patients	n=31,970	KDIGO vs RIFLE, AKIN, CK kinetics model	sCr only	lowest during hospitalization or arithmic mean of all values in the outpatient setting (7-365 days) and comparison with estimated values and values acquired by multiple imputation	-KDIGO: 0.3mg/dl increase within 48h or sCr x 1.5 within 7 days - AKIN: within 48h - RIFLE: within 7 days	**AKI:** -KDIGO:18.3% -RIFLE:16.1% - AKIN:16.6% - CK: 7% **Hospital mortality:** - KDIGO:OR 2.8(2.2-3.6) - RIFLE:OR 2.9(2.2-3.6) - AKIN:OR 2.6(2.0-3.3) - CK:OR 5.2(4.1-6.6)
Fujii/2014/ CJASN[102]	retrospective	Hospita-lized patients	n=49,185	KDIGO vs RIFLE,AKIN	sCr only	most recent value before admission or an estimated value, or the lowest sCr value within 7 days for the first 7 days. After day 8, the reference value was the lowest sCr value within the last 7 days	during hospitalization	**AKI:** - KDIGO:11.6% - RIFLE:11% - AKIN:4.8% **Hospital mortality:** - KDIGO:AUC ROC 0.78 - RIFLE:AUC ROC 0.77 - AKIN:AUC ROC 0.69

1.2.3 Pathophysiology of septic AKI

There is a lot of controversy about the pathogenesis of septic AKI and the role of renal hypoperfusion vs inflammation cascades in this phenomenon.[8;103-114] Although sepsis is characterized by a hyperdynamic state with high cardiac output, low vascular resistance and hypotension, it remains unknown how this translates to the kidney.[115,116] Schrier et al[117] state that renal vasoconstriction is the hallmark of septic AKI (Figure 1), however several reports demonstrate an increase rather than a decrease in renal blood flow (RBF) in sepsis.[118;119] Animal studies also show opposite findings.[120;121] Langenberg et al demonstrated in a model of sustained hyperdynamic sepsis in Merino sheep that septic ARF was associated with a marked increase in RBF with renal vasodilatation.[121] However, Benes et al induced sepsis in pigs and found opposite findings with an increase in renal vascular resistance, accompanied by a reduction in RBF.[120] Overall, 2/3 of the animal studies reported a decrease in RBF whereas the remaining 38% reported no change or an increase in RBF.[122]

The role of altered blood flow in the pathogenesis of septic AKI in humans remains difficult to characterize because of limitations associated with the measurement of renal blood flow during sepsis.[123] Using PAH clearance to estimate RBF in sepsis is problematic since the PAH exctraction rate is decreased and renal vein sampling is an invasive technique, often not feasible in critically ill and unstable patients. To the best of our knowledge, only three studies measured renal blood flow in septic humans, all of them suggesting increased renal blood flow.[118;119;124] Nevertheless, even when there is no decrease in RBF, there can still be a low perfusion pressure with a dysregulation of pre- en post glomerular capillary perfusion pressure, which determines renal vascular resistance.

Figure 1: Hemodynamic factors in sepsis (adapted from Schrier et al.)[117]



More recently, Prowle et al used Cine phase-contrast magnetic resonance imaging[125] to measure renal blood flow and cardiac output in 10 adult patients with established septic acute kidney injury (nine received RRT) and 11 normal volunteers.[126] They found that renal blood flow was constantly reduced as a fraction of cardiac output in established acute kidney injury and lower than in normal individuals. Renal vascular resistance was generally increased, but remained significantly correlated with systemic vascular resistance. Authors found no correlation between RBF and GFR as estimated by CrCl.[126]

However, in recent literature more and more emphasis is put on tubular damage caused by inflammatory cascades, oxidative stress, microcirculatory changes and immunological processes as the underlying pathophysiological mechanism for septic AKI as opposed to renal hypoperfusion.[127-133]

1.2.4 Animal models

The failure to successfully translate results from animals to humans[134;135] has been attributed to specific disease characteristics of sepsis (complexity and heterogeneity), inappropriate clinical trials with inadequate trial designs and the use of animal models that do not fully mimic human sepsis.

Animal models need to reproduce the complexity of human sepsis and its treatment in the ICU. Sepsis models can be divided into three categories: 1) injection of an exogenous toxin (e.g.LPS), 2) alteration of the animal's endogenous protective barrier such as intestinal leakage (e.g. cecal ligation and puncture (CLP) or colon ascendens stent peritonitis (CASP) and 3) infusion or instillation of exogenous bacteria (Table 5). The problem with LPS injection is that it causes much earlier and higher peak levels of cytokine expression compared with levels observed in human sepsis. CLP induced sepsis models show a cytokine profile similar to that in human sepsis. CLP induced sepsis increases lymphocyte apoptosis, which mimics immunosuppression at the later phase of human sepsis. But although the standard CLP model encompasses more clinical features and drug responses of human sepsis than the LPS model, it is still missing some key features, especially kidney and lung injury. Also, models such als CLP or CASP are helpful in understanding polymicrobial sepsis but human sepsis may also be caused by a single pathogen. Opposite to CLP or CASP models, bacterial infusion models may also be caused by a single pathogen. They can approximate introduction of a single pathogen in a controlled manner, allowing reproducible infection and can be translated to larger animals for the study of systemic and organ-specific hemodynamics. Animal models of sepsis differ from human sepsis because of age, comorbidity and use of supportive therapy. Animals should receive treatment comparable to the supportive treatment that is standard for ICU patients. Clinical sepsis and sepsis induced AKI are dramatically influenced by underlying diseases, which partly explains why simple animal models of sepsis do not mimic human sepsis and thus are not able to predict human response to therapeutics.[109] Studying septic AKI in animal models also requires that animals live long enough so that they can develop AKI.

Table 5: Different animals models of sepsis.[109]

Animal model	Advantage	Disadvantage
LPS Injection	Simple, sterile; some similarities with human pathophysiology	Early and transient increase in inflammatory mediators more intense than in human sepsis
CLP or CASP	Early silent period; moderate and delayed peak of mediators; multiple bacterial flora	Age and strain variability; early hemodynamic period in some models
Clinically relevant CLP	Replication of clinical risk factors	Difficulty in analyzing pathophysiological pathyways
Infusion or instillation of exogenous bacteria	Early hyperdynamic state	No change in intrarenal microcirculation; need large animals; labor intensive

Complex animal models of human sepsis may be pharmacologically more relevant than simple animal models for the testing of therapeutics, as they may ultimately predict human drug responsiveness more accurately. Despite a focus on the proinflammatory aspects of sepsis, most deaths in sepsis occur from nosocomial infections during a late prolonged immunosuppressed state, even in the face of succesful early, supportive therapies. Septic patients have defects in innate and adaptive immunity, including altered monocyte antigen presentation, decreased lymphocyte proliferation and responsiveness, and lymphocyte apoptosis and anergy. Simple CLP models demonstrate splenic apoptosis but animals generally die too early before late immunosuppression fully develops. More complex models, such as 2 hit models have therefore been developed. Other issues in model development to be considered include that host susceptibility to pathogenic factors is species dependent. For instance, rodents are much less sensitive to LPS than humans.

1.2.5 Cost and Quality of life in patients with (septic) AKI

AKI is associated with increased cost vs no-AKI across different settings. Chertow et al[73] demonstrated that, in hospitalized patients, a serum creatinine increase of 0.5 mg/dl was associated with a nearly 7,500$ increase in hospital costs across a broad spectrum of conditions. Kerr et al[136] demonstrated that AKI is associated with QALY (Quality Adjusted Life Years) loss and that the financial burden of AKI is substantial. Total postoperative costs after cardiac surgery were higher in patients with AKI compared to the matched controls and they increased progressively with the severity of AKI as determined by RIFLE stages.[137] Also in general ICU costs are higher in patients whose course is complicated by AKI.[138] In a study by Van Berendoncks et al, AKI hospital survivors were visited at home between 1 and 2 years after hospital discharge to determine morbidity, comorbidity and quality of life.[139] Authors found that the Physical Component Summary (PCS) declined and that the Mental Component Summary remained stable in relation to the US population. These results were also confirmed by comparing them to the general population values of the neighboring

countries.[139] Apart from age, other parameters such as severity of disease and clinical parameters during hospitalization did not show any significant relationship with the summary scores, suggesting that Health Related Quality of Life is difficult to predict from data available at the time of acute illness, as was found by others.[140] The need to take into account the prehospitalisation Health Related Quality of Life when examining outcomes in critically ill patients was also demonstrated by Iribarren-Diarasarri et al and Wehler et al.[141;142] Some studies have reported reduced quality of life after RRT [143-145] while others have reported no difference in long-term quality of life between RRT and no-RRT patients.[69;146] In a retrospective study of 979 ARF patients treated with RRT, 77.7% of hospital survivors responded to a questionnaire about their quality of life. 57% appeared to be self-sustaining and 49% stated that their quality of life had improved.[147] Loss of energy and limitations of physical mobility assessed by the Nottingham Health Profile were the most frequently reported complaints at six months in patients treated with RRT during their hospital stay. Functional ability as assessed by the Daily Living score was fairly good at six months.[67] Gopal et al found that in the majority of patients who survived to be discharged from hospital after combined acute multiple organ and renal failure, the overall state of health and quality of life seemed acceptable.[31] In a retrospective cohort of 24,906 critically ill patients, Vaara et al found that at six months, patients treated with RRT (6.8%) perceived their health as good as non-RRT patients by VAS (visual analogue scale).[69] Although Johansen et al found a more unfavorable Healt Related Quality of Life compared with previous reports, this might be attributed to the fact that Quality of Life was only assessed at 60 days and that included patients were generally sicker.[148] The cost per ARF survivor in the study by Korkeila et al[67] was estimated at 80,000$ per patient and in the study by Gopal et al[31], the cost for each year of survival was estimated at 50,000$ per patient.[149] Most patients felt that their treatment was worthwhile and that they would undergo the same treatment again if necessary[31], which was confirmed by several other studies.[140;143;150;151] Hamel et al estimated the cost per QALY at 128,200$ in ARF patients treated with RRT.[145] In a study by Ahlström et al the total costs of hospital treatment were 28,000$ per ARF patient treated with RRT and 222,000$/QALY for the first year.[143] Laukkanen et al[152] showed that the cost utility of acute RRT is generally poor. However, it seems to be acceptable in patients with renal recovery who survive over a year, and in 5 year survivors the cost utility ratio was around 20,000 €/QALY. However, the cost utility decreased with increasing age exceeding 1 million€/QALY in the older groups.[152] Thus cost utility of acute RRT was good in patients who had no chronic renal disease before hospitalization and whose renal function recovered.[152] There is controversy on the economic burden of CRRT (Continuous Renal Replacement Therapy) versus IRRT (Intermittent Renal Replacement Therapy) with most studies concluding that continuous techniques are more expensive without significant improve in QALY.[153-155] Etghen et al [156] however, concluded that initial CRRT is cost effective compared to IRRT by reducing the rate of long-term dialysis dependence among critically ill AKI survivors. However, a recent Cochrane review[157] does not demonstrate a benefit of CRRT over IRRT. Cost consideration also varies among centers, mainly due to differences in nurse staffing, use of fluids, anticoagulation and extracorporeal circuit. [158]

Altogether, these findings suggest that the cost and effort associated with RRT and ICU care in these patients are high but broadly comparable to those associated with the care of other serious illnesses.[67;149]

1.3 Biomarkers

1.3.1 Definition

A biomarker can be defined as a measurable or assessable entity that provides diagnostic, prognostic or treatment orienting information which can drive patient care.[159] An ideal validated biomarker should be able to detect a disease or condition non-invasively and has to be specific for the corresponding disease, and therefore should unambiguously discriminate it from disease-related changes or other disease entities.[160] An ideal biomarker should come from a readily attainable source and the result of the biomarker assessment should lead to a noticeable benefit for the patient through intervention based on the interpretation of the biomarker value, such as survival or quality of life improvement.[159]

1.3.2 Use of biomarkers in different settings

The search for biomarkers is a top priority research item in many settings such as cardiovascular diseases[161], oncology[162;163], infectious diseases[164] and renal diseases.[165;166] The aim is to allow for earlier diagnosis, even in a stage where the disease is subclinical and/or to monitor evolution under treatment.

Renal diseases are ideally suited for biomarker research given that urine is an easy accessible biofluid and its protein content is derived mainly from the kidney.[167] There has been extensive research to find new biomarkers in several domains of kidney diseases such as diabetic nephropathy, lupus nephritis, FSGS, membranous glomeronephritis, IgA nephropathy, allograft rejection in kidney transplants and Acute Kidney Injury. [165;166]

The goal of biomarker research in kidney disease is to allow for 1) earlier diagnosis of kidney injury and/or dysfunction which is particularly important because the currently used markers, such as serum creatinine, are unreliable and delay diagnosis, 2) differentiation between distinct pathological entities, 3) selection of patients who would benefit from immunosuppressive therapy, 4) selection of patients who need more RAS blockade.[167]

Especially in the field of AKI there has been an increasing interest in serum and urinary biomarkers. Serum creatinine, currently used to diagnose AKI, is an imperfect parameter that is influenced by several non-renal factors such as age, gender, race and muscle mass and only increases slowly after a renal insult causing a delay in diagnosis.[90] The potential interest of new biomarkers not only lays in earlier diagnosis but also in he differentiation between so called ‘prerenal azotemia’, a condition assumed not to be associated with tubular injury and ‘intrinsic AKI’, which is associated with tubular injury. Serum creatinine is a functional parameter that does not directly relate to tubular injury. This has made the

search for urinary biomarkers only influenced by tubular damage, a top priority in the field of nephrology (Table 6).

Table 6: Summary of the most studied biomarkers for diagnosis of AKI and their main source

Acronym/abbreviation	Legend	Main source
AP	alkaline phosphatase	Liver, bone, intestine, placenta, brush border proximal convoluted tubules
α_1MG	alpha 1 microglobulin	Liver. Reabsorption by renal proximal tubular cells
$\alpha_1$acidGP	alpha 1 acid glycoprotein	Liver. Reabsorption by renal proximal tubular cells
AST	Aspartate aminotransferase	Liver, heart, muscle, brain, red blood cells, kidney
B_2MG	beta 2 microglobulin	All nucleated cells. Reabsorption by renal proximal tubular cells
Cystatin C	Cystatin C	All nucleated cells. Reabsorption by renal proximal tubular cells
FENA	Fractional Excretion of Sodium	NA
FEUrea	Fractional Excretion of Urea	NA
GGTP	Gamma glutamyl transpeptidase	All cells except myocytes. Mainly liver and kidney (brush border proximal convoluted tubules and loop of Henle)
GST-α	Alpha glutathione S transferase	Expressed in almost all tissues. Kidney: Proximal tubular cells (cytoplasmatic)
GST-π	Pi glutathione S transferase	Expressed in almost all tissues. Kidney: Distal tubular cells (cytoplasmatic)
HGF	Hepatocyte Growth Factor	Mesenchymal cells
ICAM-1	Intracellular Adhesion Molecule 1	Endothelial cells, leukocytes
IL-6	Interleukin 6	T lymphocytes, macrophages, endothelial cells, monocytes
IL-8	Interleukin 8	Monocytes, macrophages, epithelial cells, endothelial cells
IL-10	Interleukin 10	Monocytes, lymphocytes, macrophages
IL-18	Interleukin 18	Monocytes, dendritic cells, macrophages and epithelial cells
KIM-1	Kidney Injury Molecule 1	Kidney: Proximal tubular cells
LDH	Lactate dehydrogenase	Heart, liver, red blood cells, muscle, brain, lung. Kidney: proximal tubular cells (cytoplasmatic)
LFABP	Liver type Fatty Acid Binding Protein	Hepatocytes, Kidney: proximal tubular cells

Acronym/abbreviation	Legend	Main source
NGAL	Neutrophil Gelatinase Associated Lipocalin	Leucocytes, loop of Henle and collecting ducts
NAG	N Acetyl beta Glucosaminidase	Several tissues (Liver, brain, spleen,…). Kidney: Proximal tubular cells (lysosomal)
PAI-1	Plasminogen Activator Inhibitor 1	Endothelium
PCX	Podocalyxin	Podocytes
RBP	Retinol Binding Protein	Liver. Reabsorption by renal proximal tubula cells
sTNFR-I	Soluble Tumor Necrosis Factor Receptor I	Most cells and tissues (cytotoxic, apoptotic and proinflammatory effects)
sTNFR-II	Soluble Tumor Necrosis Factor Receptor II	Most cells and tissues (proliferative and anti-apoptotic effects)
TNFα	Tumor Necrosis Factor alpha	Macrophages, lymfoïd cells, renal parenchymal cells
11k-TXB_2	11-keto-Thromboxane B_2	Platelets
vWF	Von Willebrand Factor	Endothelium, megakaryocytes, subendothelial connective tissue

Although initial results with new biomarkers were promising[168;169], these succesful results were mainly obtained in specific settings with a known timing of renal injury (e.g. cardiac surgery) and in homogenous patient populations with little comorbidities (e.g. paediactrics). Results are far less promising in adult heterogenous populations even when the timing of the renal event is known[170] but especially when the latter is unknown, multifactorial or repetitive (e.g. sepsis, critically ill).[171] Many of these markers are also influenced by other disease states such as inflammation[172] which can explain the important overlap in biomarker levels between AKI and no AKI, limiting their use as a discriminatory tool in the individual patient and questioning their additional role on top of currently used parameters such as a serum creatinine and urinary output.

1.3.3 Proteomics

Proteomics refers to the large scale study of proteins and their function and structure with the objective to find biomarkers than can help to discriminate healthy from disease affected individuals. Proteomics can potentially capture dynamic changes in protein expression, integrating both genetic and epigenetic factors.[173] Commonality in the excretion patterns of certain proteins or their fragments in various diseases offer critical insights into the default mechanisms of pathological processes.

Multiple distinct protein isoforms can be created from the same gene (thus genomics are not helpful in the differentiation of these isoforms, which might have different functions) and also, proteins can be susceptible to posttranslational modificatons.[167]

Both protein isoforms and posttranslational modifications are only detectable by studying the protein directly and can be indicative of diverse protein functions.[167] Proteomic studies can be either discovery based or targeted. Discovery based proteomics are either broad (comparison of 2 types of samples, qualitative or quantitative) or focused (protein-protein interactions). Targeted proteomic studies are based on previous knowledge of the candidate proteins of interest, and consists of different types of samples, using SR (Selected Reaction) or MR (Multiple Reaction) monitoring.[167]

Mass spectrometry (MS) is widely used as a method for protein identification. There are two main components of a mass spectrometer, namely, the ionization technique (to volatilize and ionize proteins and peptides for mass analysis with high sensitivity and specificity) and the mass analyzer. The most commonly used ionization techniques are Matrix-Assisted Laser Desorption Ionization (MALDI) and electrospray ionization (ESI). There are four different types of mass analyzers: Time of Flight (TOF), quadrupole (Q), ion trap (IT), and Fourier transform ion cyclotron resonance (FTICR).[174]
However, several caveats should be kept in mind with proteomic research. Serum proteomics is hampered by the complexity of the serum proteome containing proteins with concentration varying over 12 orders of magnitude. Therefore blood samples must be depleted of high-abundance proteins. In urinary proteomics, the high salt content and changing physicochemical properties and cellular components affect its protein concentration and the stability of proteins.[160] Sample collection and preparation should be standardized. Often, there is a lack of uniform method for sample collection and analysis. However, urine composition is influenced by diet, timing of collection, exercise, sex and age and therefore, differences in the timing of collection, the methodology used for urine concentration (specific gravity or urinary creatinine) and protein isolation, will yield distinct proteins. Also, there are still some unresolved technical issues such as the wide concentration range, the expense and the fact that it is time-consuming. Not taking into account these factors might result in observing protein differences that reflect technical bias and not a disease state. An additional problem with the novel renal biomarkers is that the urine contains a large dynamic range of protein concentrations and that the majority of discovered proteins is within the high abundance range and thus are not specific to one condition.[173]

1.4 Aim of the project

The aim of the current project was to: 1) compare different ways to calculate serum creatinine increase and assess whether small increases in serum creatinine are also associated with worse outcome in sepsis, as is the case in the cardiac surgery setting, 2) systematically review the value of new serum and urinary biomarkers in the diagnosis of AKI in different clinical settings (paediatrics, cardiac surgery, critically ill patients, emergency

department and contrast induced AKI), 3) explore the use of biomarkers such as NGAL, fractional excretion of sodium (FENa) and fractional excretion of urea (FEUrea) in the differentiation between transient and intrinsic septic AKI, 4) investigate the correlation between urinary and serum NGAL since serum NGAL levels are known to be influenced by inflammatory states and 5) investigate the role of renal hypoperfusion as a pathophysiological pathway in septic AKI.

To answer these questions, we prospectively included 195 patients with sepsis, severe sepsis or septic shock, admitted to the Intensive Care Unit (ICU) of the Ghent University Hospital.

1.5 Reference List

1. Matot I, Sprung CL. Definition of sepsis. *Intensive Care Med* 2001; 27 Suppl 1: S3-S9

2. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20: 864-874

3. Vandijck D, Decruyenaere JM, Blot SI. The value of sepsis definitions in daily ICU-practice. *Acta Clin Belg* 2006; 61: 220-226

4. Vincent JL, Martinez EO, Silva E. Evolving concepts in sepsis definitions. *Crit Care Clin* 2009; 25: 665-75

5. Abraham E, Matthay MA, Dinarello CA *et al*. Consensus conference definitions for sepsis, septic shock, acute lung injury, and acute respiratory distress syndrome: time for a reevaluation. *Crit Care Med* 2000; 28: 232-235

6. Dellinger RP, Levy MM, Rhodes A *et al*. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med* 2013; 41: 580-637

7. Levy MM, Fink MP, Marshall JC *et al*. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* 2003; 31: 1250-1256

8. Groeneveld AB. Pathogenesis of acute renal failure during sepsis.*Nephrology Dialysis Transplantation* 1994; 9 Suppl 4: 47-51

9. Jochimsen F, Schafer JH, Maurer A, Distler A. Impairment of renal function in medical intensive care: predictability of acute renal failure. *Crit Care Med* 1990; 18: 480-485

10. Neveu H, Kleinknecht D, Brivet F, Loirat P, Landais P. Prognostic factors in acute renal failure due to sepsis. Results of a prospective multicentre study. The French Study Group on Acute Renal Failure. *Nephrology Dialysis Transplantation* 1996; 11: 293-299

11. Schafer JH, Maurer A, Jochimsen F *et al*. Outcome prediction models on admission in a medical intensive care unit: do they predict individual outcome? *Crit Care Med* 1990; 18: 1111-1118

12. Vincent JL, Sakr Y, Sprung CL *et al*. Sepsis in European intensive care units: results of the SOAP study. *Crit Care Med* 2006; 34: 344-353

13. Brun-Buisson C, Meshaka P, Pinton P, Vallet B, EPISEPSIS Study Group. EPISEPSIS: a reappraisal of the epidemiology and outcome of severe sepsis in French intensive care units. *Intensive Care Medicine* 2004; 30: 580-588

14. Brun-Buisson C, Doyon F, Carlet J *et al*. Incidence, risk factors, and outcome of severe sepsis and septic shock in adults. A multicenter prospective study in intensive care units. French ICU Group for Severe Sepsis. *JAMA* 1995; 274: 968-974

15. Gaieski DF, Edwards JM, Kallan MJ, Carr BG. Benchmarking the incidence and mortality of severe sepsis in the United States. *Crit Care Med* 2013; 41: 1167-1174

16. Rivers E, Nguyen B, Havstad S *et al*. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* 2001; 345: 1368-1377

17. Castellanos-Ortega A, Suberviola B, Garcia-Astudillo LA *et al*. Impact of the Surviving Sepsis Campaign protocols on hospital length of stay and mortality in septic shock patients: results of a three-year follow-up quasi-experimental study. *Crit Care Med* 2010; 38: 1036-1043

18. Ferrer R, Artigas A, Suarez D *et al*. Effectiveness of treatments for severe sepsis: a prospective, multicenter, observational study. *Am J Respir Crit Care Med* 2009; 180: 861-866

19. Kumar A, Roberts D, Wood KE *et al*. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006; 34: 1589-1596

20. Levy MM, Dellinger RP, Townsend SR *et al*. The Surviving Sepsis Campaign: results of an international guideline-based performance improvement program targeting severe sepsis. *Crit Care Med* 2010; 38: 367-374

21. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. *JAMA* 2014; 311: 1308-1316

22. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001; 29: 1303-1310

23. Edbrooke DL, Hibbert CL, Kingsley JM, Smith S, Bright NM, Quinn JM. The patient-related costs of care for sepsis patients in a United Kingdom adult general intensive care unit. *Crit Care Med* 1999; 27: 1760-1767

24. Weycker D, Akhras KS, Edelsberg J, Angus DC, Oster G. Long-term mortality and medical care charges in patients with severe sepsis. *Crit Care Med* 2003; 31: 2316-2323

25. Letarte J, Longo CJ, Pelletier J, Nabonne B, Fisher HN. Patient characteristics and costs of severe sepsis and septic shock in Quebec. *J Crit Care* 2002; 17: 39-49

26. Moerer O, Burchardi H. [The cost of sepsis]. *Anaesthesist* 2006; 55 Suppl 1: 36-42

27. Dellinger RP, Carlet JM, Masur H *et al*. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. *Crit Care Med* 2004; 32: 858-873

28. Levy MM, Pronovost PJ, Dellinger RP *et al*. Sepsis change bundles: converting guidelines into meaningful change in behavior and clinical outcome. *Crit Care Med* 2004; 32: S595-S597

29. Talmor D, Greenberg D, Howell MD, Lisbon A, Novack V, Shapiro N. The costs and cost-effectiveness of an integrated sepsis treatment protocol. *Crit Care Med* 2008; 36: 1168-1174

30. Capuzzo M, Moreno RP, Jordan B, Bauer P, Alvisi R, Metnitz PG. Predictors of early recovery of health status after intensive care. *Intensive Care Med* 2006; 32: 1832-1838

31. Gopal I, Bhonagiri S, Ronco C, Bellomo R. Out of hospital outcome and quality of life in survivors of combined acute multiple organ and renal failure treated with continuous venovenous hemofiltration/hemodiafiltration. *Intensive Care Med* 1997; 23: 766-772

32. Fletcher SN, Kennedy DD, Ghosh IR *et al*. Persistent neuromuscular and neurophysiologic abnormalities in long-term survivors of prolonged critical illness. *Crit Care Med* 2003; 31: 1012-1016

33. Garnacho-Montero J, Madrazo-Osuna J, Garcia-Garmendia JL *et al*. Critical illness polyneuropathy: risk factors and clinical consequences. A cohort study in septic patients. *Intensive Care Med* 2001; 27: 1288-1296

34. Granja C, Dias C, Costa-Pereira A, Sarmento A. Quality of life of survivors from severe sepsis and septic shock may be similar to that of others who survive critical illness. *Crit Care* 2004; 8: R91-R98

35. Semmler A, Widmann CN, Okulla T *et al*. Persistent cognitive impairment, hippocampal atrophy and EEG changes in sepsis survivors. *J Neurol Neurosurg Psychiatry* 2013; 84: 62-69

36. Tennila A, Salmi T, Pettila V, Roine RO, Varpula T, Takkunen O. Early signs of critical illness polyneuropathy in ICU patients with systemic inflammatory response syndrome or sepsis. *Intensive Care Med* 2000; 26: 1360-1363

37. Haraldsen P, Andersson R. Quality of life, morbidity, and mortality after surgical intensive care: a follow-up study of patients treated for abdominal sepsis in the surgical intensive care unit. *Eur J Surg Suppl* 2003; 23-27

38. Heyland DK, Hopman W, Coo H, Tranmer J, McColl MA. Long-term health-related quality of life in survivors of sepsis. Short Form 36: a valid and reliable measure of health-related quality of life. *Crit Care Med* 2000; 28: 3599-3605

39. Hofhuis JG, Spronk PE, van Stel HF, Schrijvers GJ, Rommes JH, Bakker J. The impact of critical illness on perceived health-related quality of life during ICU treatment, hospital stay, and after hospital discharge: a long-term follow-up study. *Chest* 2008; 133: 377-385

40. Karlsson S, Varpula M, Ruokonen E *et al*. Incidence, treatment, and outcome of severe sepsis in ICU-treated adults in Finland: the Finnsepsis study. *Intensive Care Med* 2007; 33: 435-443

41. Korosec JH, Jagodic K, Podbregar M. Long-term outcome and quality of life of patients treated in surgical intensive care: a comparison between sepsis and trauma. *Crit Care* 2006; 10: R134

42. Longo CJ, Heyland DK, Fisher HN, Fowler RA, Martin CM, Day AG. A long-term follow-up study investigating health-related quality of life and resource use in survivors of severe sepsis: comparison of recombinant human activated protein C with standard care. *Crit Care* 2007; 11: R128

43. McLauchlan GJ, Anderson ID, Grant IS, Fearon KC. Outcome of patients with abdominal sepsis treated in an intensive care unit. *Br J Surg* 1995; 82: 524-529

44. Perl TM, Dvorak L, Hwang T, Wenzel RP. Long-term survival and function after suspected gram-negative sepsis. *JAMA* 1995; 274: 338-345

45. Paratz JD, Kenardy J, Mitchell G *et al*. IMPOSE (IMProving Outcomes after Sepsis)-the effect of a multidisciplinary follow-up service on health-related quality of life in patients postsepsis syndromes-a double-blinded randomised controlled trial: protocol. *BMJ Open* 2014; 4: 1-6

46. Schmidt K, Thiel P, Mueller F *et al*. Sepsis survivors monitoring and coordination in outpatient health care (SMOOTH): study protocol for a randomized controlled trial. *Trials* 2014; 15: 283

47. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348: 1546-1554

48. Wang HE, Shapiro NI, Angus DC, Yealy DM. National estimates of severe sepsis in United States emergency departments. *Crit Care Med* 2007; 35: 1928-1936

49. Dombrovskiy VY, Martin AA, Sunderram J, Paz HL. Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003. *Crit Care Med* 2007; 35: 1244-1250

50. Wilhelms SB, Huss FR, Granath G, Sjoberg F. Assessment of incidence of severe sepsis in Sweden using different ways of abstracting International Classification of Diseases codes: difficulties with methods and interpretation of results. *Crit Care Med* 2010; 38: 1442-1449

51. Flaatten H. Epidemiology of sepsis in Norway in 1999. *Crit Care* 2004; 8: R180-R184

52. Lagu T, Rothberg MB, Shieh MS, Pekow PS, Steingrub JS, Lindenauer PK. Hospitalizations, costs, and outcomes of severe sepsis in the United States 2003 to 2007. *Crit Care Med* 2012; 40: 754-761

53. Bagshaw SM, Uchino S, Bellomo R *et al*. Septic acute kidney injury in critically ill patients: clinical characteristics and outcomes. *Clinical Journal of The American Society of Nephrology: CJASN* 2007; 2: 431-439

54. Prescott GJ, Metcalfe W, Baharani J *et al*. A prospective national study of acute renal failure treated with RRT: incidence, aetiology and outcomes. *Nephrol Dial Transplant* 2007; 22: 2513-2519

55. Bagshaw SM, George C, Bellomo R. Early acute kidney injury and sepsis: a multicentre evaluation. *Crit Care* 2008; 12: 1-9

56. Brivet FG, Kleinknecht DJ, Loirat P, Landais PJ. Acute renal failure in intensive care units--causes, outcome, and prognostic factors of hospital mortality; a prospective, multicenter study. French Study Group on Acute Renal Failure. *Crit Care Med* 1996; 24: 192-198

57. Uchino S, Kellum JA, Bellomo R *et al*. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA* 2005; 294: 813-818

58. Carl DE, Grossman C, Behnke M, Sessler CN, Gehr TW. Effect of timing of dialysis on mortality in critically ill, septic patients with acute renal failure. *Hemodial Int* 2010; 14: 11-17

59. Chou YH, Huang TM, Wu VC *et al*. Impact of timing of renal replacement therapy initiation on outcome of septic acute kidney injury. *Crit Care* 2011; 15: 1-9

60. Ali T, Khan I, Simpson W *et al*. Incidence and outcomes in acute kidney injury: a comprehensive population-based study. *J Am Soc Nephrol* 2007; 18: 1292-1298

61. Brochard L, Abroug F, Brenner M *et al*. An Official ATS/ERS/ESICM/SCCM/SRLF Statement: Prevention and Management of Acute Renal Failure in the ICU Patient: an international consensus conference in intensive care medicine. *Am J Respir Crit Care Med* 2010; 181: 1128-1155

62. Wald R, Quinn RR, Adhikari NK *et al*. Risk of chronic dialysis and death following acute kidney injury. *Am J Med* 2012; 125: 585-593

63. Bellomo R, Cass A, Cole L *et al*. Intensity of continuous renal-replacement therapy in critically ill patients. *N Engl J Med* 2009; 361: 1627-1638

64. Cole L, Bellomo R, Silvester W, Reeves JH. A prospective, multicenter study of the epidemiology, management, and outcome of severe acute renal failure in a "closed" ICU system. *Am J Respir Crit Care Med* 2000; 162: 191-196

65. Feest TG, Round A, Hamad S. Incidence of severe acute renal failure in adults: results of a community based study. *BMJ* 1993; 306: 481-483

66. Khan IH, Catto GR, Edward N, MacLeod AM. Acute renal failure: factors influencing nephrology referral and outcome. *Qjm* 1997; 90: 781-785

67. Korkeila M, Ruokonen E, Takala J. Costs of care, long-term prognosis and quality of life in patients requiring renal replacement therapy during intensive care. *Intensive Care Medicine* 2000; 26: 1824-1831

68. Liano F, Junco E, Pascual J, Madero R, Verde E. The spectrum of acute renal failure in the intensive care unit compared with that seen in other settings. The Madrid Acute Renal Failure Study Group. *Kidney Int Suppl* 1998; 66: S16-S24

69. Vaara ST, Pettila V, Reinikainen M, Kaukonen KM. Population-based incidence, mortality and quality of life in critically ill patients treated with renal replacement therapy: a nationwide retrospective cohort study in Finnish intensive care units. *Crit Care* 2012; 16: 1-8

70. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; 8: R204-R212

71. Mehta RL, Kellum JA, Shah SV *et al*. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 2007; 11: 1-8

72. Brown JR, Cochran RP, Dacey LJ *et al*. Perioperative increases in serum creatinine are predictive of increased 90-day mortality after coronary artery bypass graft surgery. *Circulation* 2006; 114: I409-I413

73. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005; 16: 3365-3370

74. Gottlieb SS, Abraham W, Butler J *et al*. The prognostic importance of different definitions of worsening renal function in congestive heart failure. *J Card Fail* 2002; 8: 136-141

75. Lassnigg A, Schmid ER, Hiesmayr M *et al*. Impact of minimal increases in serum creatinine on outcome in patients after cardiothoracic surgery: do we have to revise current definitions of acute renal failure? *Crit Care Med* 2008; 36: 1129-1137

76. Loef BG, Epema AH, Smilde TD *et al*. Immediate postoperative renal function deterioration in cardiac surgical patients predicts in-hospital mortality and long-term survival. *J Am Soc Nephrol* 2005; 16: 195-200

77. Newsome BB, Warnock DG, McClellan WM *et al*. Long-term risk of mortality and end-stage renal disease among the elderly after small increases in serum creatinine level during hospitalization for acute myocardial infarction. *Arch Intern Med* 2008; 168: 609-616

78. Ryckwaert F, Alric P, Picot MC, Djoufelkit K, Colson P. Incidence and circumstances of serum creatinine increase after abdominal aortic surgery. *Intensive Care Med* 2003; 29: 1821-1824

79. Smith GL, Vaccarino V, Kosiborod M *et al*. Worsening renal function: what is a clinically meaningful change in creatinine during hospitalization with heart failure? *J Card Fail* 2003; 9: 13-25

80. Kidney disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Int Suppl* 2012; 2: 1-138

81. Fliser D, Laville M, Covic A *et al*. A European Renal Best Practice (ERBP) position statement on the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines on acute kidney injury: part 1: definitions, conservative management and contrast-induced nephropathy. *Nephrol Dial Transplant* 2012; 27: 4263-4272

82. James M, Bouchard J, Ho J *et al*. Canadian Society of Nephrology commentary on the 2012 KDIGO clinical practice guideline for acute kidney injury. *Am J Kidney Dis* 2013; 61: 673-685

83. Lewington A, Kanagasundaram S. Renal Association Clinical Practice Guidelines on acute kidney injury. *Nephron Clin Pract* 2011; 118 Suppl 1: c349-c390

84. Palevsky PM, Liu KD, Brophy PD *et al*. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for acute kidney injury. *Am J Kidney Dis* 2013; 61: 649-672

85. Bagshaw SM, George C, Bellomo R. A comparison of the RIFLE and AKIN criteria for acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2008; 23: 1569-1574

86. Joannidis M, Metnitz B, Bauer P *et al*. Acute kidney injury in critically ill patients classified by AKIN versus RIFLE using the SAPS 3 database. *Intensive Care Med* 2009; 35: 1692-1702

87. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. *Kidney Int* 2008; 73: 538-546

88. Siew ED, Matheny ME, Ikizler TA *et al*. Commonly used surrogates for baseline renal function affect the classification and prognosis of acute kidney injury. *Kidney Int* 2010; 77: 536-542

89. Zavada J, Hoste E, Cartin-Ceba R *et al*. A comparison of three methods to estimate baseline creatinine for RIFLE classification. *Nephrol Dial Transplant* 2010; 25: 3911-3918

90. Endre ZH, Pickering JW, Walker RJ. Clearance and beyond: the complementary roles of GFR measurement and injury biomarkers in acute kidney injury (AKI). *Am J Physiol Renal Physiol* 2011; 301: F697-F707

91. Lopes JA, Fernandes P, Jorge S *et al*. Acute kidney injury in intensive care unit patients: a comparison between the RIFLE and the Acute Kidney Injury Network classifications. *Crit Care* 2008; 12: R110

92. Haase M, Bellomo R, Matalanis G, Calzavacca P, Dragun D, Haase-Fielitz A. A comparison of the RIFLE and Acute Kidney Injury Network classifications for cardiac surgery-associated acute kidney injury: a prospective cohort study. *J Thorac Cardiovasc Surg* 2009; 138: 1370-1376

93. Robert AM, Kramer RS, Dacey LJ *et al*. Cardiac surgery-associated acute kidney injury: a comparison of two consensus criteria. *Ann Thorac Surg* 2010; 90: 1939-1943

94. Yan X, Jia S, Meng X *et al*. Acute kidney injury in adult postcardiotomy patients with extracorporeal membrane oxygenation: evaluation of the RIFLE classification and the Acute Kidney Injury Network criteria. *Eur J Cardiothorac Surg* 2010; 37: 334-338

95. Chang CH, Lin CY, Tian YC *et al*. Acute kidney injury classification: comparison of AKIN and RIFLE criteria. *Shock* 2010; 33: 247-252

96. Englberger L, Suri RM, Li Z *et al*. Clinical accuracy of RIFLE and Acute Kidney Injury Network (AKIN) criteria for acute kidney injury in patients undergoing cardiac surgery. *Crit Care* 2011; 15: 1-9

97. Wang HE, Jain G, Glassock RJ, Warnock DG. Comparison of absolute serum creatinine changes versus Kidney Disease: Improving Global Outcomes consensus definitions for characterizing stages of acute kidney injury. *Nephrol Dial Transplant* 2013; 28: 1447-1454

98. Roy AK, Mc GC, Treacy C *et al*. A Comparison of Traditional and Novel Definitions (RIFLE, AKIN, and KDIGO) of Acute Kidney Injury for the Prediction of Outcomes in Acute Decompensated Heart Failure. *Cardiorenal Med* 2013; 3: 26-37

99. Rodrigues FB, Bruetto RG, Torres US, Otaviano AP, Zanetta DM, Burdmann EA. Incidence and mortality of acute kidney injury after myocardial infarction: a comparison between KDIGO and RIFLE criteria. *PLoS One* 2013; 8: 1-8

100. Bastin AJ, Ostermann M, Slack AJ, Diller GP, Finney SJ, Evans TW. Acute kidney injury after cardiac surgery according to Risk/Injury/Failure/Loss/End-stage, Acute Kidney Injury Network, and Kidney Disease: Improving Global Outcomes classifications. *J Crit Care* 2013; 28: 389-396

101. Zeng X, McMahon GM, Brunelli SM, Bates DW, Waikar SS. Incidence, outcomes, and comparisons across definitions of AKI in hospitalized individuals. *Clin J Am Soc Nephrol* 2014; 9: 12-20

102. Fujii T, Uchino S, Takinami M, Bellomo R. Validation of the Kidney Disease Improving Global Outcomes criteria for AKI and comparison of three criteria in hospitalized patients. *Clin J Am Soc Nephrol* 2014; 9: 848-854

103. Bock HA. Pathophysiology of acute renal failure in septic shock: from prerenal to renal failure. [Review] [37 refs]. *Kidney International - Supplement* 1998; 64: S15-S18

104. Bougle A, Duranteau J. Pathophysiology of sepsis-induced acute kidney injury: the role of global renal blood flow and renal vascular resistance. *Contributions to Nephrology* 2011; 174: 89-97

105. Wan L, Bellomo R, Di GD, Ronco C. The pathogenesis of septic acute renal failure. *Curr Opin Crit Care* 2003; 9: 496-502

106. Badr KF. Sepsis-associated renal vasoconstriction: potential targets for future therapy. *Am J Kidney Dis* 1992; 20: 207-213

107. Chawla LS, Dommu A, Berger A, Shih S, Patel SS. Urinary sediment cast scoring index for acute kidney injury: a pilot study. *Nephron* 2008; 110: c145-c150

108. Di G, May CN, Bellomo R. Vital organ blood flow during hyperdynamic sepsis. *Chest* 2003; 124: 1053-1059

109. Doi K, Leelahavanichkul A, Yuen PS, Star RA. Animal models of sepsis and sepsis-induced kidney injury. *J Clin Invest* 2009; 119: 2868-2878

110. Perazella MA, Coca SG, Kanbay M, Brewster UC, Parikh CR. Diagnostic value of urine microscopy for differential diagnosis of acute kidney injury in hospitalized patients. *Clin J Am Soc Nephrol* 2008; 3: 1615-1619

111. Perazella MA, Parikh CR. How can urine microscopy influence the differential diagnosis of AKI? *Clinical Journal of The American Society of Nephrology: CJASN* 2009; 4: 691-693

112. Perazella MA, Coca SG, Hall IE, Iyanam U, Koraishy M, Parikh CR. Urine microscopy is associated with severity and worsening of acute kidney injury in hospitalized patients. *Clinical Journal of The American Society of Nephrology: CJASN* 2010; 5: 402-408

113. Thijs A, Thijs LG. Pathogenesis of renal failure in sepsis. *Kidney Int Suppl* 1998; 66: S34-S37

114. Zarjou A, Agarwal A. Sepsis and acute kidney injury. *J Am Soc Nephrol* 2011; 22: 999-1006

115. Badr KF. Novel mediators of sepsis-associated renal failure. *Semin Nephrol* 1994; 14: 3-7

116. Kelleher SP, Robinette JB, Conger JD. Sympathetic nervous system in the loss of autoregulation in acute renal failure. *American Journal of Physiology* 1984; 246: F379-F386

117. Schrier RW, Wang W. Acute renal failure and sepsis. *N Engl J Med* 2004; 351: 159-169

118. Lucas CE, Rector FE, Werner M, Rosenberg IK. Altered renal homeostasis with acute sepsis. Clinical significance. *Archives of Surgery* 1973; 106: 444-449

119. Rector F, Goyal SC, Rosenberg IK, Lucas CE. Renal hyperemia in association with clinical sepsis. *Surgical Forum* 1972; 23: 51-53

120. Benes J, Chvojka J, Sykora R *et al*. Searching for mechanisms that matter in early septic acute kidney injury: an experimental study. *Critical Care (London, England)* 2011; 15: 1-14

121. Langenberg C, Wan L, Egi M, May CN, Bellomo R. Renal blood flow in experimental septic acute renal failure. *Kidney International* 2006; 69: 1996-2002

122. Langenberg C, Bellomo R, May C, Wan L, Egi M, Morgera S. Renal blood flow in sepsis. *Crit Care* 2005; 9: R363-R374

123. Prowle JR, Ishikawa K, May CN, Bellomo R. Renal blood flow during acute renal failure in man. *Blood Purif* 2009; 28: 216-225

124. Brenner M, Schaer GL, Mallory DL, Suffredini AF, Parrillo JE. Detection of renal blood flow abnormalities in septic and critically ill patients using a newly designed indwelling thermodilution renal vein catheter. *Chest* 1990; 98: 170-179

125. Prowle JR, Molan MP, Hornsey E, Bellomo R. Cine phase-contrast magnetic resonance imaging for the measurement of renal blood flow. *Contributions to Nephrology* 2010; 165: 329-336

126. Prowle JR, Molan MP, Hornsey E, Bellomo R. Measurement of renal blood flow by phase-contrast magnetic resonance imaging during septic acute kidney injury: a pilot investigation. *Crit Care Med* 2012; 40: 1768-1776

127. Le Dorze M., Legrand M, Payen D, Ince C. The role of the microcirculation in acute kidney injury. *Curr Opin Crit Care* 2009; 15: 503-508

128. Frithiof R. Sepsis-induced acute kidney injury-is there a lack of energy? *Intensive Care Med* 2012; 38: 735-737

129. Heemskerk S, Pickkers P, Bouw MP *et al*. Upregulation of renal inducible nitric oxide synthase during human endotoxemia and sepsis is associated with proximal tubule injury. *Clin J Am Soc Nephrol* 2006; 1: 853-862

130. Himmelfarb J, McMonagle E, Freedman S *et al*. Oxidative stress is increased in critically ill patients with acute renal failure. *Journal of the American Society of Nephrology* 2004; 15: 2449-2456

131. Wang Z, Holthoff JH, Seely KA *et al*. Development of oxidative stress in the peritubular capillary microenvironment mediates sepsis-induced renal microcirculatory failure and acute kidney injury. *American Journal of Pathology* 2012; 180: 505-516

132. Wu L, Gokden N, Mayeux PR. Evidence for the role of reactive nitrogen species in polymicrobial sepsis-induced renal peritubular capillary dysfunction and tubular injury. *J Am Soc Nephrol* 2007; 18: 1807-1815

133. Zarjou A, Agarwal A. Sepsis and acute kidney injury. *Journal of the American Society of Nephrology* 2011; 22: 999-1006

134. Allgren RL, Marbury TC, Rahman SN *et al*. Anaritide in acute tubular necrosis. Auriculin Anaritide Acute Renal Failure Study Group. *N Engl J Med* 1997; 336: 828-834

135. Hirschberg R, Kopple J, Lipsett P *et al*. Multicenter clinical trial of recombinant human insulin-like growth factor I in patients with acute renal failure. *Kidney Int* 1999; 55: 2423-2432

136. Kerr M, Bedford M, Matthews B, O'Donoghue D. The economic impact of acute kidney injury in England. *Nephrol Dial Transplant* 2014; 29: 1362-1368

137. Dasta JF, Kane-Gill SL, Durtschi AJ, Pathak DS, Kellum JA. Costs and outcomes of acute kidney injury (AKI) following cardiac surgery. *Nephrol Dial Transplant* 2008; 23: 1970-1974

138. Vandijck DM, Oeyen S, Decruyenaere JM, Annemans L, Hoste EA. Acute kidney injury, length of stay, and costs in patients hospitalized in the intensive care unit. *Acta Clin Belg Suppl* 2007; 341-345

139. Van Berendoncks AM, Elseviers MM, Lins RL. Outcome of acute kidney injury with different treatment options: long-term follow-up. *Clin J Am Soc Nephrol* 2010; 5: 1755-1762

140. Maynard SE, Whittle J, Chelluri L, Arnold R. Quality of life and dialysis decisions in critically ill patients with acute renal failure. *Intensive Care Med* 2003; 29: 1589-1593

141. Wehler M, Geise A, Hadzionerovic D *et al*. Health-related quality of life of patients with multiple organ dysfunction: individual changes and comparison with normative population. *Crit Care Med* 2003; 31: 1094-1101

142. Iribarren-Diarasarri S, Aizpuru-Barandiaran F, Munoz-Martinez T *et al*. Health-related quality of life as a prognostic factor of survival in critically ill patients. *Intensive Care Med* 2009; 35: 833-839

143. Ahlstrom A, Tallgren M, Peltonen S, Rasanen P, Pettila V. Survival and quality of life of patients requiring acute renal replacement therapy. *Intensive Care Med* 2005; 31: 1222-1228

144. Desai AA, Baras J, Berk BB *et al*. Management of acute kidney injury in the intensive care unit: a cost-effectiveness analysis of daily vs alternate-day hemodialysis. *Arch Intern Med* 2008; 168: 1761-1767

145. Hamel MB, Phillips RS, Davis RB *et al*. Outcomes and cost-effectiveness of initiating dialysis and continuing aggressive care in seriously ill hospitalized adults. SUPPORT Investigators. Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatments. *Ann Intern Med* 1997; 127: 195-202

146. Landoni G, Zangrillo A, Franco A *et al*. Long-term outcome of patients who require renal replacement therapy after cardiac surgery. *Eur J Anaesthesiol* 2006; 23: 17-22

147. Morgera S, Kraft AK, Siebert G, Luft FC, Neumayer HH. Long-term outcomes in acute renal failure patients treated with continuous renal replacement therapies. *American Journal of Kidney Diseases* 2002; 40: 275-279

148. Johansen KL, Smith MW, Unruh ML, Siroka AM, O'Connor TZ, Palevsky PM. Predictors of health utility among 60-day survivors of acute kidney injury in the Veterans Affairs/National Institutes of Health Acute Renal Failure Trial Network Study. *Clin J Am Soc Nephrol* 2010; 5: 1366-1372

149. Gopal I, Bhonagiri S, Ronco C, Bellomo R. Out of hospital outcome and quality of life in survivors of combined acute multiple organ and renal failure treated with continuous venovenous hemofiltration/hemodiafiltration. *Intensive Care Med* 1997; 23: 766-772

150. Delannoy B, Floccard B, Thiolliere F *et al*. Six-month outcome in acute kidney injury requiring renal replacement therapy in the ICU: a multicentre prospective study. *Intensive Care Med* 2009; 35: 1907-1915

151. Rimes-Stigare C, Awad A, Martensson J, Martling CR, Bell M. Long-term outcome after acute renal replacement therapy: a narrative review. *Acta Anaesthesiol Scand* 2012; 56: 138-146

152. Laukkanen A, Emaus L, Pettila V, Kaukonen KM. Five-year cost-utility analysis of acute renal replacement therapy: a societal perspective. *Intensive Care Med* 2013; 39: 406-413

153. Manns B, Doig CJ, Lee H *et al*. Cost of acute renal failure requiring dialysis in the intensive care unit: clinical and resource implications of renal recovery. *Crit Care Med* 2003; 31: 449-455

154. De Smedt DM, Elseviers MM, Lins RL, Annemans L. Economic evaluation of different treatment modalities in acute kidney injury. *Nephrol Dial Transplant* 2012; 27: 4095-4101

155. Klarenbach S, Manns B, Pannu N, Clement FM, Wiebe N, Tonelli M. Economic evaluation of continuous renal replacement therapy in acute renal failure. *Int J Technol Assess Health Care* 2009; 25: 331-338

156. Ethgen O, Schneider AG, Bagshaw SM, Bellomo R, Kellum JA. Economics of dialysis dependence following renal replacement therapy for critically ill acute kidney injury patients. *Nephrol Dial Transplant* 2014;

157. Rabindranath K, Adams J, MacLeod AM, Muirhead N. Intermittent versus continuous renal replacement therapy for acute renal failure in adults. *Cochrane Database Syst Rev* 2007; 1-51

158. Srisawat N, Lawsin L, Uchino S, Bellomo R, Kellum JA. Cost of acute renal replacement therapy in the intensive care unit: results from The Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) study. *Crit Care* 2010; 14: 1-10

159. Kohn EC, Azad N, Annunziata C, Dhamoon AS, Whiteley G. Proteomics as a tool for biomarker discovery. *Dis Markers* 2007; 23: 411-417

160. Kienzl-Wagner K, Pratschke J, Brandacher G. Proteomics--a blessing or a curse? Application of proteomics technology to transplant medicine. *Transplantation* 2011; 92: 499-509

161. Wang TJ. Assessing the role of circulating, genetic, and imaging biomarkers in cardiovascular risk prediction. *Circulation* 2011; 123: 551-565

162. Bichsel VE, Liotta LA, Petricoin EF, III. Cancer proteomics: from biomarker discovery to signal pathway profiling. *Cancer J* 2001; 7: 69-78

163. Hanke JH, Webster KR, Ronco LV. Protein biomarkers and drug design for cancer treatments. *Eur J Cancer Prev* 2004; 13: 297-305

164. Chen SC, Kontoyiannis DP. New molecular and surrogate biomarker-based tests in the diagnosis of bacterial and fungal infection in febrile neutropenic patients. *Curr Opin Infect Dis* 2010; 23: 567-577

165. Fliser D, Novak J, Thongboonkerd V *et al*. Advances in urinary proteome analysis and biomarker discovery. *J Am Soc Nephrol* 2007; 18: 1057-1071

166. Goligorsky MS, Addabbo F, O'Riordan E. Diagnostic potential of urine proteome: a broken mirror of renal diseases. *J Am Soc Nephrol* 2007; 18: 2233-2239

167. Konvalinka A, Scholey JW, Diamandis EP. Searching for new biomarkers of renal diseases through proteomics. *Clin Chem* 2012; 58: 353-365

168. Mishra J, Dent C, Tarabishi R *et al*. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet* 2005; 365: 1231-1238

169. Parikh CR, Mishra J, Thiessen-Philbrook H *et al*. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. *Kidney Int* 2006; 70: 199-203

170. Parikh CR, Coca SG, Thiessen-Philbrook H *et al*. Postoperative biomarkers predict acute kidney injury and poor outcomes after adult cardiac surgery. *J Am Soc Nephrol* 2011; 22: 1748-1757

171. Siew ED, Ikizler TA, Gebretsadik T *et al*. Elevated urinary IL-18 levels at the time of ICU admission predict adverse clinical outcomes. *Clin J Am Soc Nephrol* 2010; 5: 1497-1505

172. Giasson J, Li GH, Chen Y. Neutrophil gelatinase-associated lipocalin (NGAL) as a new biomarker for non--acute kidney injury (AKI) diseases. *Inflamm Allergy Drug Targets* 2011; 10: 272-282

173. Mischak H, Apweiler R, Banks RE *et al*. Clinical proteomics: A need to define the field and to begin to set adequate standards. *Proteomics Clin Appl* 2007; 1: 148-156

174. Wu J, Chen YD, Gu W. Urinary proteomics as a novel tool for biomarker discovery in kidney diseases. *J Zhejiang Univ Sci B* 2010; 11: 227-237

CHAPTER 2:

PROGNOSTIC ROBUSTNESS OF CURRENT AKI DEFINITIONS IN PATIENTS WITH SEPSIS.

Jill Vanmassenhove, MD.[1], Norbert Lameire, MD, PhD[1], Annemieke Dhondt, MD, PhD.[1], Raymond Vanholder, MD, PhD.[1], Wim Van Biesen, MD, PhD.[1]

[1]Renal Division, Ghent University Hospital

Paper currently under submission

2.1 Abstract

Objective: To evaluate whether modifications in the definitions of how to calculate change of serum creatinine (sCr) and in the cut-off value, alter the prognostic value of AKI for prediction of mortality at 3 months, 1 and 2 years.

Design: A prospective observational cohort study in patients admitted to ICU with sepsis.

Setting: Single-centre university hospital.

Patients: 195 septic patients were included between 12/01/2010 and 27/03/2011. The prognostic value of the diagnosis "AKI" was evaluated by using three different methods to calculate the change of sCr: either focusing on the onset status by determining the difference between the highest value in the 24 hours after ICU admission and a pre-admission historical baseline value (ΔHIS) or an estimated baseline value (ΔEST), or by subtracting the value after 24 hours of ICU from the admission value (ΔADM). For each of these definitions, different cut-off levels of sCr increase (0.1, 0.2, 0.3, 0.4 and 0.5 mg/dl), were evaluated.

Interventions: none

Measurements and main results: Mortality at 3 months, 1 and 2 years in AKI defined as ΔADM>0.3mg/dl was 48.1%, 63.0% and 63.0% vs 27.7%,39.8% and 47.6% in no AKI respectively (OR(95%CI):2.42(1.06-5.54),2.58(1.11-5.97) and 1.87(0.81-4.33); 0.3 mg/dl was the lowest cut-off value that was discriminatory. When AKI was defined as ΔHIS>0.3mg/dl or ΔEST>0.3mg/dl, there was no significant difference in mortality between AKI and no AKI. After adjusting for severity of illness, even ΔADM>0.3mg/dl was no longer independently associated with mortality.

Conclusion: To predict mortality in sepsis patients, the AKI diagnostic criterion of an increase of sCr>0.3mg/dl should be calculated referring to the ICU admission value, allowing to incorporate the impact of initial therapy, rather than as the difference between the highest Scr and a known or estimated historical baseline value.

2.2 Introduction

There is growing evidence that Acute Kidney Injury (AKI) is an independent predictor for mortality rather than an innocent bystander. It has been demonstrated, especially in cardiac surgery, that even small increases in serum creatinine (sCr) are associated with increased mortality risk.[1-4] It is however unclear whether this also applies to more complex multifactorial conditions such as sepsis where the impact of AKI might be overwhelmed by the severity of the underlying disease. In such circumstances, AKI could rather be a marker of severity of illness than a cause of negative outcome. About 45 to 70% of AKI in the Intensive Care Unit (ICU) is associated with sepsis and septic AKI can be considered as a separate clinical entity with a largely unknown pathophysiology, and possibly different prognosis from non-septic AKI.[5]

Despite recent advances in the uniformisation of the definition of AKI, there is continuing debate on the exact interpretation of the definitions[6-9], and different definitions, incorrectly presented as being KDIGO or AKIN, continue to appear in the literature.[10-13] The most

important issue in this debate relates to the method to calculate the increase of sCr. The currently proposed practice to use the highest creatinine value in a certain time-period after ICU admission does not take into account the evolution of sCr after starting therapeutic interventions. As such, the current definitions do not allow to distinguish the potential difference in prognosis for an AKI patient who is admitted with a high, but decreasing sCr value, e.g. a traditionally called prerenal AKI, and a patient who's sCr increases progressively despite therapy. This might be problematic for the interpretation of future randomised controlled trials exploring interventions to prevent or treat AKI, as they might become false negative by including a mix of patients with a different prognosis.

Also, the cut off value of a sCr increase of 0.3mg/dl, as proposed by AKIN, KDIGO and ERBP [6;8;9], has not previously been validated in a specific cohort of sepsis patients.

In this prospective cohort study we wanted to assess short and longer term mortality in a cohort of sepsis patients admitted to the ICU in the Ghent University Hospital. We evaluated the impact on the prognostic value of AKI, the latter diagnosed by three different ways to calculate the change of sCr. The first took into account the evolution of sCr in the first 24 hours after initiation of therapy, whereas the two others followed the more classical approach. In addition we intended to explore the robustness of the currently proposed increase in sCr of 0.3mg/dl as cut-off in sepsis, by comparing it's predictive value to that of either smaller or larger increases.

2.3 Materials and methods

One hundred and ninety five consecutive adult patients (age ≥ 17 years) with sepsis admitted to the intensive care unit (ICU) of the Ghent University Hospital between 12/01/2010 and 27/03/2011 were included. Sepsis, severe sepsis and septic shock were defined according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference guidelines.[14] Exclusion criteria were: a history of liver and/or kidney transplantation, ICU stay less than 24 hours and patients treated with chronic hemodialysis.

The criterion of increase of sCr as definition of AKI was modified by all possible combinations of two interventions:

1° by using three different ways to calculate the change of sCr: either **A/** as the highest value within 24 hours after ICU admission minus the value of a pre-admission historical baseline (ΔHIS); **B/** as the highest value 24 hours after ICU admission minus an estimated baseline value obtained by solving the MDRD equation assuming a GFR of 75 ml/min/1.73m², as suggested by ADQI[7] (ΔEST); **C/** as the value at 24 hours after admission minus the value at ICU admission (ΔADM), thus capturing the evolution of sCr after start of therapy; of note, the first two calculations by definition cannot capture a potential decrease of Scr by therapeutic interventions.

2° by changing the absolute value of the increase of sCr (0.1, 0.2, 0.3, 0.4, and 0.5 mg/dl), to assess the robustness of the 0.3 mg/dl increase criterion in this sepsis population. Blood samples for measuring sCr were collected during the first five days after ICU admission, centrifuged immediately and frozen at -80° for later batch analysis, using an Isotope Dilution Mass Spectroscopy traceable method (Roche Diagnostics®).

Survival status was ascertained based on hospital records, or by a telephone interview with the family practitioner performed by JV.

Statistical analysis: Results are reported as medians and interquartile ranges (IQR) for continuous variables, unless otherwise specified. Discrete variables are reported as numbers and/or percentages. All statistical analyses were performed using SPSS® 19.

Chi square was used to asses a difference in prevalence in case of a dichotomous outcome. Results are reported as Odds ratio with 95% confidence intervals (OR;95%CI). Student t test was used to compare the means of normally distributed continuous variables whereas non parametric tests were used to compare the medians of not normally distributed continuous variables.

Logistic regression (3 months) and Cox regression (1 and 2 year) were performed in forward and backward mode, presenting age, gender, presence of pre-existing renal insufficiency (eGFR<60ml/min)(CKD), APACHE II score and need for ventilation to the model as potential predictors for mortality. As additional parameter, AKI according to the above specified definitions with different cut offs (0.1, 0.2, 0.3, 0.4 and 0.5 mg/dl) of increase of sCr and different ways to calculate the increase of sCr (ΔADM, ΔHIS and ΔEST) was added as a dummy code to the model. The effect of adding a small increase of sCr on predictive performance of mortality was assessed by comparing AUC ROC analysis of the different models with and without the different definitions of AKI, as applied in this study.

For the short term (3 month) mortality, we used a logistic regression model, as in this case, actual duration of survival was not considered relevant and mortality was approached in a dichotomous way. For the longer term survival (1 and 2 year), we considered survival time as a continuous variable rather than a dichotomous one, and therefore, in that setting, Cox regression was performed.

2.4 Results

Descriptive patient data

In this study, 195 consecutive patients with sepsis, severe sepsis and septic shock and admitted to ICU between 12/01/2010 and 27/03/2011 were included. Nine (4.6%), 63 (32.3%) and 123 (63.1%) had sepsis, severe sepsis and septic shock, respectively. Overall mortality rates at the ICU, at three months, 1 year and 2 years after admission, were 23.1%, 31.3%, 43.6% and 50.3%, respectively. Of the patients who needed RRT during their ICU stay (n=27(13.8%)), cumulative mortality rate during ICU stay was 55.6% (OR 5.75(2.44-13.53). Eighty-three percent of patients who were treated with RRT and survived ICU (n=10/12), survived up to two years. One patient died at three months and another died at year 1.

Demographics and relevant data for AKI vs no AKI according to the three different definitions (ΔADM>0.3, ΔHIS>0.3 and ΔEST>0.3) are presented in Table 1.

The prevalence of AKI varied according to the definition used. Based on ΔADM>0.3 vs ΔHIS>0.3 and ΔEST>0.3, 27(13.8%) vs 98(50.3%) and 89(45.6) patients were labelled as AKI (p<0.001).

Patients classified in the AKI vs no AKI group according to ΔADM>0.3 had a greater severity of illness as demonstrated by a higher APACHE II score (p=0.003), a higher need for invasive ventilation (p<0.001) and a longer ICU stay (p=0.009). When ΔHIS or ΔEST were used, there was a difference in APACHE II score in AKI vs no AKI but not in need for invasive ventilation or length of ICU stay (Table 1). The difference between historical and admission sCr was larger in the patients with AKI defined by ΔHIS or ΔEST, as compared to ΔADM (p<0.001). Based on ΔHIS or ΔEST, there is a steady decrease in sCr vs the admission value in the AKI group over the following four days, as opposed to AKI according to ΔADM (Figure 1A, Figure 1B and Figure 1C).

Mortality in AKI vs no AKI according to different definitions

In patients with AKI vs no AKI defined according to ΔADM>0.3, ICU mortality, and three months, 1 year and 2 years mortality rates were 44.4%,48.1%,63% and 63% vs 18.7%,27.7%,39.8% and 47.6%, respectively (OR 3.48(1.48-8.18), 2.42(1.06-5.54), 2.58(1.11-5.97) and 1.87(0.81-4.33)) (Table 1 and Figure 2A).

When AKI was defined according to ΔHIS>0.3 or ΔEST>0.3, mortality rates were not different between AKI and no-AKI respectively at any of the time points (Table 1 and Figure 2A).

Mortality in AKI vs no AKI in ICU survivors, according to ΔADM,ΔHIS and ΔEST> 0.3

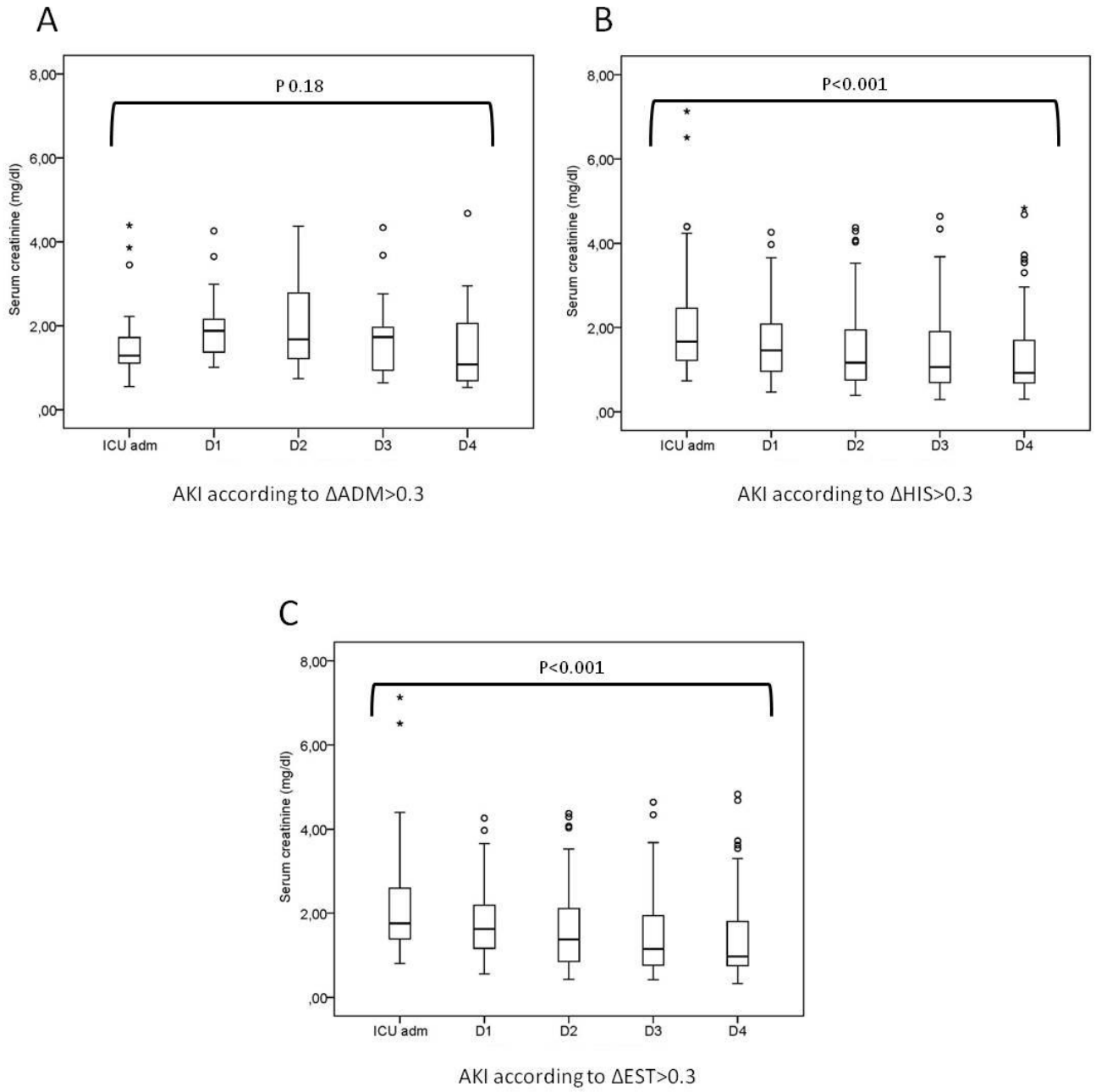
In ICU survivors (n=150), mortality rates at three months, 1 year and 2 years in AKI vs no-AKI according to ΔADM>0.3 were 6.7%, 33.3% and 33.3% vs 11.9%, 25.9% and 35.6% respectively (OR 0.53(0.07-4.32), 1.43(0.46-4.47) and 0.91(0.29-2.81) respectively). Based on ΔHIS>0.3, mortality rates were 9.6%, 23.3% and 30.1% vs 13%, 29.9% and 40.3% respectively (OR 0.71(0.26-1.98), 0.71(0.34-1.48) and 0.64(0.33-1.26)). Based on ΔEST>0.3 these were 13.4%, 28.4% and 31.3% vs 9.6%, 25.3% and 38.6% in AKI vs no AKI respectively (OR 1.46(0.53-4.00), 1.17(0.57-2.42) and 0.73(0.37-1.44)) (Figure 2B).

Table 1: Demographics in AKI vs no-AKI according to different definitions.

	ΔADM>0.3			**ΔHIS>0.3**			**ΔEST>0.3**		
AKI/noAKI (n/%)	AKI (27/13.8)	no AKI (166/85.1)	p value	AKI(98/50.3)	no AKI (97/49.7)	p value	AKI (89/45.6)	no AKI (106/54.4)	p value
%male	66.7	61.4	0.6	67.3	57.7	0.17	65.2	60.4	0.49
Age(y)(mean/sd)	66.2(10.7)	60.7(15.6)	0.08	62.6(14.1)	60.2(15.8)	0.28	65.1(13.5)	58.2(15.5)	0.001
APACHE II	27(9)	22(9)	0.003	25(8)	19(9)	<0.001	25(8)	20(9)	<0.001
Ventilation(%)	92.6	48.2	<0.001	60.2	48.5	0.10	61.8	48.1	0.06
CKD(%)	25.9	13.9	0.11	18.4	12.4	0.25	28.1	4.7	<0.001
ICU admission serum creatinine (mg/dl)	1.29(0.66)	1.05(0.89)	0.01	1.67(1.25)	0.79(0.40)	<0.001	1.76(1.24)	0.80(0.39)	<0.001
Historical baseline creatinine (mg/dl)	0.98(0.41)	0.83(0.32)	0.18	0.88(0.37)	0.82(0.34)	0.52	0.98(0.35)	0.79(0.32)	0.001
RRT need(%)	51.9	7.8	<0.001	23.5	4.1	<0.001	27	2.8	<0.001
ICU LOS(d) in ICU survivors	15(22)	6(8)	0.009	6(9)	5(10)	0.34	7(9)	6(9)	0.24
ICU Mort(%)	44.4	18.7	0.003	25.5	20.6	0.42	24.7	21.7	0.62
Mort at three months(%)	48.1	27.7	0.03	32.7	39.9	0.68	33.7	29.2	0.50
Mort at 1 year(%)	63	39.8	0.024	42.9	44.3	0.84	46.1	41.5	0.52
Mort at 2 years(%)	63	47.6	0.14	48	52.6	0.52	48.3	51.9	0.62

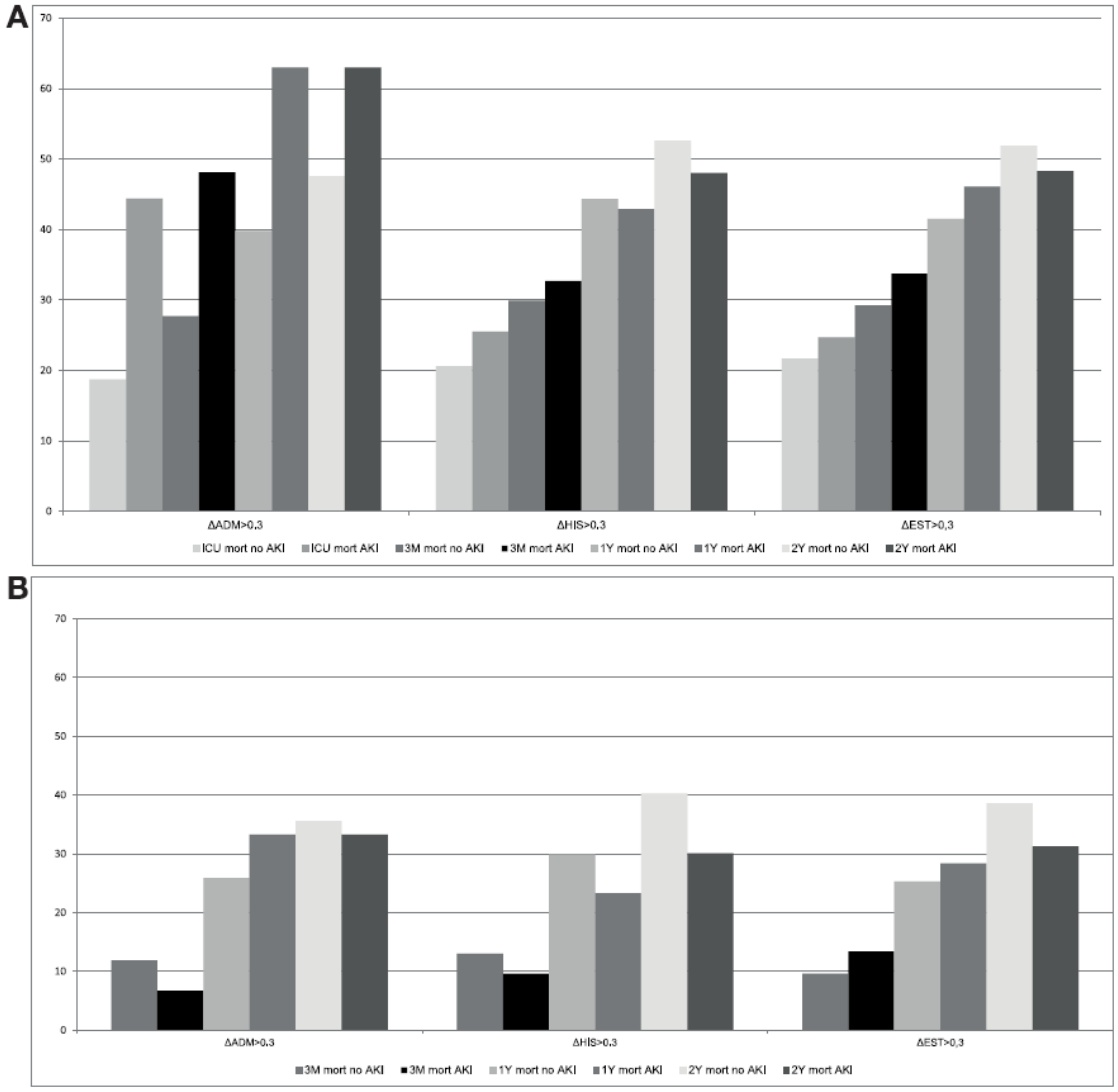
AKI: Acute Kidney Injury, ΔADM>0.3= Serum creatinine increase > 0.3 mg/dl based on the difference between the value 24 hours after admission and ICU admission, ΔHIS>0.3=Serum creatinine increase > 0.3 mg/dl based on the difference between the highest value during the first 24 hours after ICU admission and a historical baseline value, ΔEST>0.3= Serum creatinine increase > 0.3 mg/dl based on the difference between the highest value during the first 24 hours after ICU admission and an estimated baseline value, APACHE II: Acute Physiology and Chronic Health Evaluation II score, CKD: Chronic Kidney Disease, RRT: Renal Replacement Therapy, ICU LOS: Intensive Care Unit Length of Stay, Mort: Mortality

Figure 1: Evolution of serum creatinine over 4 days in patients with Acute Kidney Injury, according to different definitions.



*Legend Figure 1: As opposed to Acute Kidney Injury based on ΔADM>0.3**(A)**, there is a steady decrease in serum creatinine after admission over the following four days when AKI is defined according to ΔHIS>0.3**(B)** or ΔEST>0.3**(C)**.*

Figure 2: Mortality rates (%) in Acute Kidney Injury vs no Acute Kidney Injury according to different definitions, in the entire cohort and in ICU survivors separately.



*Legend Figure 2: **A**: Mortality rates at ICU, 3 months, 1 year and 2 years in the entire cohort in Acute Kidney Injury vs no Acute Kidney Injury, either based on ΔADM>0.3, ΔHIS>0.3 or ΔEST>0.3. Only based on ΔADM>0.3, there is a higher mortality at ICU, 3 months and 1 year in AKI vs no AKI. At year 2, there is no significant different in mortality between AKI and no AKI with either of the definitions. **B**: Mortality rates at 3 months, 1 year and 2 years in ICU survivors in Acute Kidney Injury vs no Acute Kidney Injury, either based on ΔADM>0.3, ΔHIS>0.3 or ΔEST>0.3. There is no significant difference in mortality between Acute Kidney Injury and no Acute Kidney Injury at the three time points, independent of the definition used.*

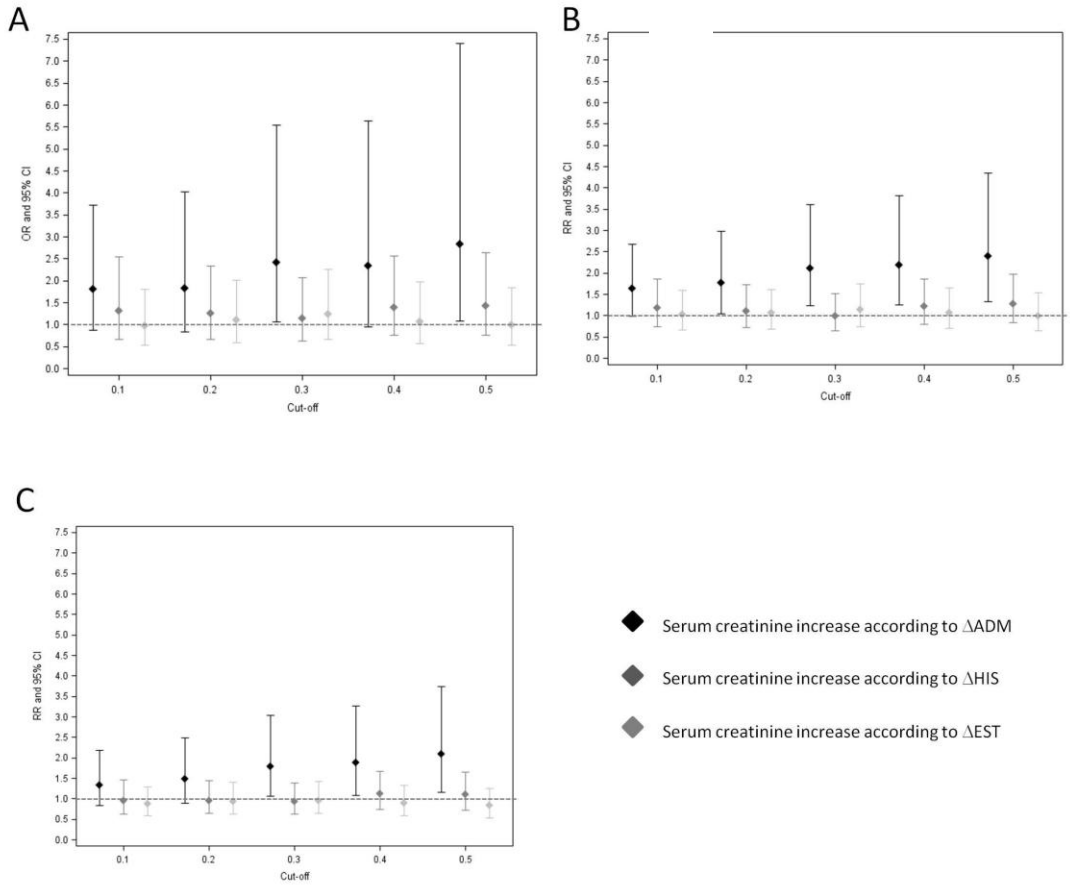
Thus with none of the above definitions (ΔADM>0.3, ΔHIS>0.3 or ΔEST>0.3) there was a significant difference in mortality rates between AKI and no AKI in septic patients who survived their ICU stay, either at three months, 1 year and 2 years.

Also other variants of calculating ΔsCr were assessed, which confirmed that in sepsis patients only the evolution of sCr as compared to admission sCr was prognostic for mortality (supplemental table 1, 2 and 3).

Prognostic value for mortality of different cut-off values for serum creatinine increase

Using the different combinations of how to calculate increase of sCr (ΔADM, ΔHIS, ΔEST) and different cut-off levels for that increase (0.1mg/dl to 0.5mg/dl with increments of 0.1mg/dl), we found that in univariate analysis an increase in sCr of 0.3mg/dl was the lowest robust cut-off value that was still associated with mortality at three months in the entire cohort (OR 2.42, 95% CI 1.06-5.54), but only if the difference in increase of sCr was based on ΔADM (Figure 3A). At year 1 and 2 an increase in sCr of 0.3mg/dl was also the lowest robust cut-off for prediction of mortality in the entire cohort, again only when the definition is based on ΔADM (RR 2.11(1.24-3.6) and RR 1.79(1.06-3.03) for mortality at 1 year and 2 years respectively) (Figure 3B and Figure 3C).

Figure 3: Odds ratio of incremental cut-off values for serum creatinine increase and mortality in the entire cohort.



*Legend figure 3: A 0.3 mg/dl increase in serum creatinine is the lowest robust cut-off value associated with 3 months mortality **(A)**, but only if this increase is based on ΔADM>0.3 (OR 2.42(1.06-5.54). At year 1 **(B)** and year 2 **(C)**, a serum creatinine increase of 0.3 mg/dl is also het lowest robust cut-off value associated with mortality but again only if this increase is based on ΔADM>0.3 (RR 2.11(1.24-3.6) and RR 1.79(1.06-303) at year 1 and year 2 respectively).*

Multivariate analysis for prediction of mortality

In a logistic regression model adjusted for age and gender, APACHE II score (OR per point: 1.06, 95% CI 1.01-1.11) and Need for Ventilation (OR 2.89, 95% CI 1.41-5.95) but not AKI according to ΔADM, were independent predictors for mortality at three months (Table 2). Comparable results were obtained when the other definitions of AKI were used (Table 2).

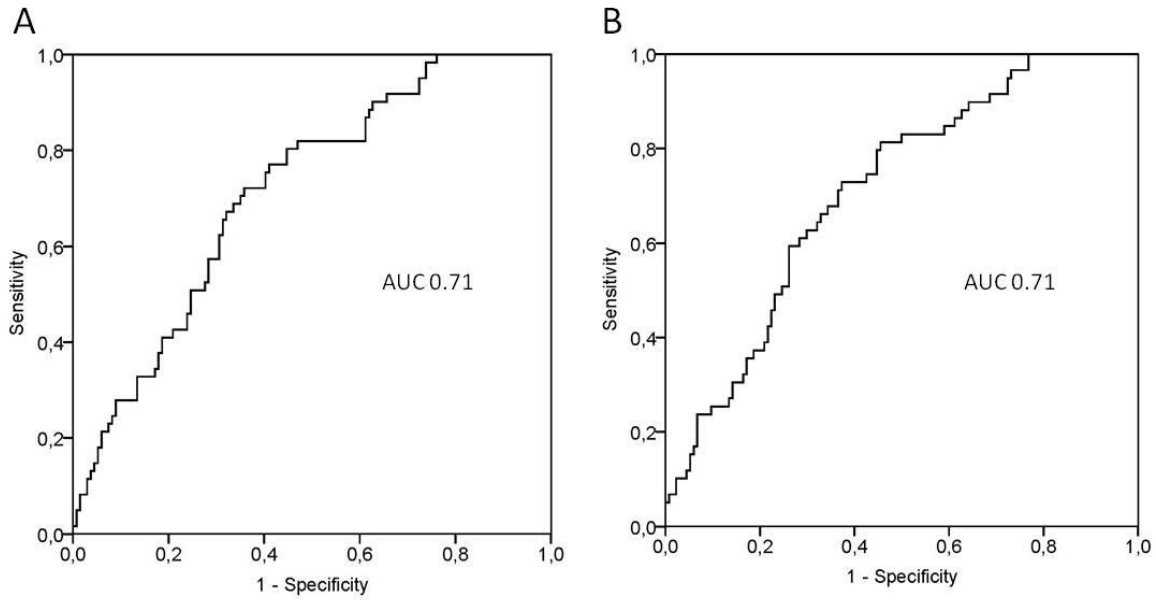
Table 2: Multivariate logistic regression for prediction of mortality at three months including different AKI definitions in the model

	Exp(B)	**95% CI**	**p value**
ΔADM>0.3	1.15	0.46-2.87	0.76
Age	1.01	0.99-1.04	0.29
Gender	0.75	0.38-1.49	0.42
APACHE II	1.06	1.01-1.11	0.02
Need for Ventilation	2.89	1.41-5.95	0.004
ΔHIS>0.3	0.62	0.30-1.26	0.18
Age	1.01	0.99-1.04	0.37
Gender	0.69	0.35-1.36	0.28
APACHE II	1.08	1.03-1.14	0.003
Need for Ventilation	2.97	1.48-5.95	0.002
ΔEST>0.3	0.64	0.31-1.31	0.22
Age	1.01	0.99-1.04	0.27
Gender	0.70	0.36-1.39	0.31
APACHE II	1.08	1.03-1.14	0.003
Need for Ventilation	2.96	1.48-5.92	0.002

ΔADM>0.3=Serum creatinine increase > 0.3 mg/dl based on the difference between the value 24 hours after admission and ICU admission, APACHE II: Acute Physiology and Chronic Health Evaluation II score, ΔHIS>0.3=Serum creatinine increase > 0.3 mg/dl based on the difference between the highest value during the first 24 hours after ICU admission and a historical baseline value, ΔEST>0.3= Serum creatinine increase > 0.3 mg/dl based on the difference between the highest value during the first 24 hours after ICU admission and an estimated baseline value.

AUC ROC analysis confirmed that ΔADM>0.3 did not add discriminatory value above APACHE II score and Need for Ventilation for prognostication of mortality at 3 months (AUC 0.71 without ΔADM>0.3 and AUC 0.71 with ΔADM>0.3) (Figure 4A and Figure 4B).

Figure 4: AUC ROC for predicting mortality at three months, either including or not including ΔADM>0.3 in the model.



Legend figure 4: **A**: AUC ROC for predicting mortality at three months for the model not including ΔADM>0.3 is 0.71. **B**:Adding ΔADM>0.3 to the model does not improve discriminative power for prediction of mortality (AUC ROC 0.71)

In a Cox regression multivariate model applied to ICU survivors, only age was an independent predictor for mortality both at 1 year (RR=1.04, 95% CI: 1.01-1.06) and 2 years (RR=1.02, 95% CI: 1.001-1.043) (Table 3). This suggests that longer term mortality in sepsis patients is more influenced by underlying comorbidity such as older age than by AKI or other parameters of acute severity of illness.

Table 3: Multivariate cox regression analysis for prediction of mortality at 1 year and 2 years in ICU survivors.

1 year mortality in ICU survivors	**Exp(B)**	**95% CI**	**p value**
Age	1.04	1.01-1.06	0.005
Creatinine day 1	0.69	0.38-1.25	0.22
CKD	1.73	0.73-4.12	0.21
ΔADM>0.3	1.38	0.43-4.40	0.59
Need for Ventilation	1.07	0.55-2.07	0.85
Gender	0.65	0.32-1.31	0.23
APACHE II	1.006	0.96-1.05	0.80
2 years mortality in ICU survivors			
Age	1.02	1.001-1.043	0.04
Creatinine day 1	0.47	0.26-0.85	0.01
CKD	2.17	0.99-4.72	0.05
ΔADM>0.3	1.17	0.38-3.60	0.78
Need for Ventilation	1.29	0.73-2.29	0.38
Gender	0.67	0.37-1.22	0.19
APACHE II	1.03	0.99-1.07	0.18

ICU: Intensive Care Unit, CKD: Chronic Kidney Disease, ΔADM>0.3= Serum creatinine increase > 0.3 mg/dl based on the difference between the value 24 hours after admission and ICU admission, APACHE II: Acute Physiology and Chronic Health Evaluation II score.

2.5 Discussion

This study demonstrates that the predictive value of AKI for mortality in sepsis only exists if the increase in sCr is calculated based on the difference between the sCr value at 24 hours and the ICU admission value. AKI in sepsis is not associated with mortality when the increase in sCr is calculated as the difference between the highest sCr or when the baseline sCr is back-calculated by solving the MDRD formula assuming a GFR of 75 ml/min/1,73m2.[7] An increase in sCr of 0.3mg/dl according to the ICU admission value is the lowest robust value still associated with mortality, confirming previous data in the cardiac surgery setting. However, when adjusted for severity of illness, this increase in sCr of 0.3mg/dl was no longer associated with mortality. Taken together, our data suggest that, at least in sepsis,

definitions of AKI are only robust to predict mortality when they do allow to assess the evolution of sCr after initiation of therapy, at least during the first 24 hours.

Although the definition of AKI has become progressively more uniform since the introduction of RIFLE [7], uncertainty and debate on the methodology to calculate the increase of sCr remains, and different interpretations are still appearing in the literature.[10-13] Most guidelines advocate the use of the difference of the highest sCr value and a pre admission historical baseline value,[15;16] or a back calculated sCr by solving the MDRD formula assuming a GFR of 75ml/min/1,73m^2 if the preadmission baseline value is unknown.[17-20] It has been demonstrated that the use of these surrogates as baseline sCr can lead to misclassification by either under- or overestimation of AKI.[15] Siew et al also investigated the impact of different surrogate baseline values in a large cohort of 4863 adults on AKI diagnosis and outcome.[16] These authors not only demonstrated that the incidence of AKI based on the ICU admission value, decreased compared to AKI defined based on a pre admission historical baseline value, as in our cohort, but that mortality rates were higher with increasing AKIN stages but were largely different according to the surrogate value that was used.[16] As in our study, mortality rates were highest when AKI was defined according to the admission value. However, Siew et al used a cohort containing mainly non-critically ill patients (only 19% of patients were admitted to ICU) and only in-hospital and 60 days mortality were evaluated, without adjustment for severity of illness.[16]

Another potential point of discussion is the use of the highest sCr value after admission as reference value. This strategy does not allow to study the impact of the evolution of sCr after admission and after start of therapeutic interventions. In our cohort, AKI was associated with mortality, but only if based on the difference between the value 24 hours after admission and the value at ICU admission. There was no association with mortality when the increase in sCr was calculated as the difference between the highest value in the 24 hours after admission and either a known historical baseline value or a value that was back-calculated assuming a GFR of 75 ml/min/1,73m2. These findings suggest that the evolution of sCr in the first 24 hours at the ICU, and thus very likely the response to fluid challenge, is more predictive for outcome than the admission value itself, which is in line with previous observations.[21] Definitions comparing the highest value after admission with a pre-existing value probably also include a relatively high number of patients with so called "functional" or prerenal AKI, who have a good response to treatment and thus a more benign prognosis. This is reflected in the fact that AKI as defined by these criteria, called ΔHIS or ΔEST in our study, was more prevalent than when defined by ΔADM, and that length of ICU stay was not different in AKI vs no AKI when based on the ΔHIS or ΔEST definition, but was much longer in AKI vs no AKI patients when defined by ΔADM.

Although it is generally accepted that AKI is associated with increased mortality[22], this topic is still a matter of debate, even in the non-critically ill. In a population based study of AKI, no association between AKI and outcome was found.[23] A higher risk for chronic dialysis need, but not mortality, was found in a large cohort of ICU survivors[24], in parallel with another study, where a 28-fold risk increase for development of CKD stage 4-5 was observed compared to an only twofold increase in mortality for patients with AKI vs no AKI.[25] Of note however is that several reports indicated that the duration of AKI rather than the diagnosis of AKI per se was highly predictive of mortality, an aspect that was not evaluated in the current study. This might explain why, in our cohort, the fact of being diagnosed with AKI, was no longer independently associated with mortality after adjustment for severity of illness. Our data on the other hand clarify something that is visualized by none of the other

definitions, i.e. that the response to treatment in the first 24 hours of observation also contributes to the prognosis, probably by unveiling those who respond to volume resuscitation. Our findings thus indicate that the response to treatment in the first 24 hours also has a contributive prognostic value. Several older and more recent studies also demonstrated that the most important predictors for mortality were already present at admission to the ICU and included advanced age, the presence of infection, a past history of chronic diseases and the presence of other failing organs.[26 27 28 29;30]

Although it was demonstrated in the recent literature that even small increases in sCr are independently associated with a higher risk of mortality, these results are mainly obtained in cohorts of cardiac surgery patients, after coronarography and after myocardial infarction[1-4;31;32,33], and can therefore not necessarily be extrapolated to other situations, such as sepsis. The latter can be considered as a complex often multifactorial variant of AKI with a largely unknown pathophysiology which in turn might translate into a different impact of AKI on mortality risk assessment. Our results confirm that, even in a cohort of sepsis patients, a 0.3mg/dl increase in sCr 24 hours after admission compared to the ICU admission value, seems to be the lowest robust threshold for increased risk.

The strong points of our prospective study are the availability of longer term outcomes and the detailed patient information that is included, and that the results were obtained in a well defined homogenous patient category (patients with sepsis admitted to ICU). More specifically, we have the historical baseline creatinine of all patients, as well as 2 year mortality data.

Our study is the first to consider that the evolution of sCr after start of therapy, rather than an absolute highest value over a time period might be important for prognostic purposes in sepsis patients.

A limitation of this study is the observational nature so that causal assumptions can not be made, but this is unavoidable in this setting. Furthermore, it is single centre, and describes only a relatively small cohort of patients. Further prospective studies on long term outcome in septic AKI are warranted.

2.6 Conclusion

In sepsis patients, the AKI diagnostic criterion of sCr increase of >0.3mg/dl should for prognostic purposes be calculated referring to the ICU admission value, rather than by the difference between a highest value and a known or estimated historical baseline value.

2.7 Reference List

1. Lassnigg A, Schmid ER, Hiesmayr M *et al*. Impact of minimal increases in serum creatinine on outcome in patients after cardiothoracic surgery: do we have to revise current definitions of acute renal failure? *Crit Care Med* 2008; 36: 1129-1137

2. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005; 16: 3365-3370

3. Newsome BB, Warnock DG, McClellan WM *et al*. Long-term risk of mortality and end-stage renal disease among the elderly after small increases in serum creatinine level during hospitalization for acute myocardial infarction. *Arch Intern Med* 2008; 168: 609-616

4. Thakar CV, Christianson A, Freyberg R, Almenoff P, Render ML. Incidence and outcomes of acute kidney injury in intensive care units: a Veterans Administration study. *Crit Care Med* 2009; 37: 2552-2558

5. Bagshaw SM, Uchino S, Bellomo R *et al*. Septic acute kidney injury in critically ill patients: clinical characteristics and outcomes. *Clinical Journal of The American Society of Nephrology: CJASN* 2007; 2: 431-439

6. Kidney disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury . *Kidney Int Suppl* 2012; 2: 1-138

7. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; 8: R204-R212

8. Fliser D, Laville M, Covic A *et al*. A European Renal Best Practice (ERBP) position statement on the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines on acute kidney injury: part 1: definitions, conservative management and contrast-induced nephropathy. *Nephrol Dial Transplant* 2012; 27: 4263-4272

9. Mehta RL, Kellum JA, Shah SV *et al*. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 2007; 11: R31

10. Fujii T, Uchino S, Takinami M, Bellomo R. Validation of the Kidney Disease Improving Global Outcomes criteria for AKI and comparison of three criteria in hospitalized patients. *Clin J Am Soc Nephrol* 2014; 9: 848-854

11. Leedahl DD, Frazee EN, Schramm GE *et al*. Derivation of Urine Output Thresholds That Identify a Very High Risk of AKI in Patients with Septic Shock. *Clin J Am Soc Nephrol* 2014;

12. Linder A, Fjell C, Levin A, Walley KR, Russell JA, Boyd JH. Small acute increases in serum creatinine are associated with decreased long-term survival in the critically ill. *Am J Respir Crit Care Med* 2014; 189: 1075-1081

13. Machado MN, Nakazone MA, Maia LN. Prognostic Value of Acute Kidney Injury after Cardiac Surgery according to Kidney Disease: Improving Global Outcomes Definition and Staging (KDIGO) Criteria. *PLoS One* 2014; 9: e98028

14. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20: 864-874

15. Zavada J, Hoste E, Cartin-Ceba R *et al*. A comparison of three methods to estimate baseline creatinine for RIFLE classification. *Nephrol Dial Transplant* 2010; 25: 3911-3918

16. Siew ED, Matheny ME, Ikizler TA *et al*. Commonly used surrogates for baseline renal function affect the classification and prognosis of acute kidney injury. *Kidney Int* 2010; 77: 536-542

17. Bagshaw SM, George C, Bellomo R. A comparison of the RIFLE and AKIN criteria for acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2008; 23: 1569-1574

18. Hoste EA, Clermont G, Kersten A *et al*. RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients: a cohort analysis. *Crit Care* 2006; 10: R73

19. Lopes JA, Fernandes P, Jorge S *et al*. Acute kidney injury in intensive care unit patients: a comparison between the RIFLE and the Acute Kidney Injury Network classifications. *Crit Care* 2008; 12: R110

20. Uchino S, Bellomo R, Goldsmith D, Bates S, Ronco C. An assessment of the RIFLE criteria for acute renal failure in hospitalized patients. *Crit Care Med* 2006; 34: 1913-1917

21. Levy MM, Macias WL, Vincent JL *et al*. Early changes in organ function predict eventual survival in severe sepsis. *Crit Care Med* 2005; 33: 2194-2201

22. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. *Kidney Int* 2008; 73: 538-546

23. Ali T, Khan I, Simpson W *et al*. Incidence and outcomes in acute kidney injury: a comprehensive population-based study. *J Am Soc Nephrol* 2007; 18: 1292-1298

24. Wald R, Quinn RR, Luo J *et al*. Chronic dialysis and death among survivors of acute kidney injury requiring dialysis. *JAMA* 2009; 302: 1179-1185

25. Lo LJ, Go AS, Chertow GM *et al*. Dialysis-requiring acute renal failure increases the risk of progressive chronic kidney disease. *Kidney Int* 2009; 76: 893-899

26. De Mendonca A, Vincent JL, Suter PM *et al*. Acute renal failure in the ICU: risk factors and outcome evaluated by the SOFA score. *Intensive Care Med* 2000; 26: 915-921

27. Pereira MB, Zanetta DM, Abdulkader RC. The real importance of pre-existing comorbidities on long-term mortality after acute kidney injury. *PLoS ONE [Electronic Resource] 7(10):e47746,* 2012;

28. Sasse KC, Nauenberg E, Long A, Anton B, Tucker HJ, Hu TW. Long-term survival after intensive care unit admission with sepsis. *Critical Care Medicine* 1995; 23: 1040-1047

29. Ponte B, Felipe C, Muriel A, Tenorio MT, Liano F. Long-term functional evolution after an acute kidney injury: a 10-year study. *Nephrology Dialysis Transplantation* 2008; 23: 3859-3866

30. Ostermann M, Chang RW. Acute kidney injury in the intensive care unit according to RIFLE. *Crit Care Med* 2007; 35: 1837-1843

31. Ishani A, Nelson D, Clothier B *et al*. The magnitude of acute serum creatinine increase after cardiac surgery and the risk of chronic kidney disease, progression of kidney disease, and death. *Arch Intern Med* 2011; 171: 226-233

32. Lafrance JP, Miller DR. Acute kidney injury associates with increased long-term mortality. *J Am Soc Nephrol* 2010; 21: 345-352

33. Weisbord SD, Chen H, Stone RA *et al*. Associations of increases in serum creatinine with mortality and length of hospital stay after coronary angiography. *Journal of the American Society of Nephrology* 2006; 17: 2871-2877

CHAPTER 3: URINARY AND SERUM BIOMARKERS FOR THE DIAGNOSIS OF ACUTE KIDNEY INJURY: AN IN DEPTH REVIEW OF THE LITERATURE.

Jill Vanmassenhove, Raymond Vanholder, Evi Nagler, Wim Van Biesen

Renal Division, University Hospital, Ghent, Belgium

Nephrology Dialysis and Transplantation,2013 Feb 28(2):254-273

3.1 Abstract

Objective: Acute Kidney Injury (AKI) remains associated with high morbidity and mortality despite progress in medical care. Although the RIFLE and AKIN criteria, based on serum creatinine (Screa) and urine output, were a step forward in diagnosing AKI, a reliable tool to differentiate between true parenchymal and prerenal azotemia in clinical practice is still lacking. In the last decade, many papers on the use of new urinary and serum biomarkers for diagnosis and prognostication of AKI have been published. Thus, the question arises which biomarker is a reliable differential diagnostic tool under which circumstances.

Design: We searched Medline from inception to April 2012 using medical subject heading and textwords for AKI and biomarkers (NGAL, KIM-1, Cystatin C, IL-6, IL-8, IL-18, NAG, glutathione transferases and LFABP) to identify relevant papers in 5 different settings (pediactrics, cardiac surgery, emergency department, critically ill, and contrast induced nephropathy).

Results: We included 87 relevant papers, reporting on 74 studies. Depending upon the setting, 7 to 27 different definitions of AKI were used. Reported diagnostic performance of the different biomarkers was variable from poor to excellent, and no consistent generalizable conclusions can be drawn on their diagnostic value.

Conclusion: Early diagnosing of AKI in clinical conditions by using new serum and urinary biomarkers remains cumbersome, especially in those settings where timing and etiology of AKI are not well defined. Putting too much emphasis on markers that have not convincingly proven reliability might lead to incorrect interpretation of clinical trials. Further research in this field is warranted before biomarkers can be introduced in clinical practice .

3.2 Introduction

Acute Kidney Injury (AKI) remains associated with high morbidity and mortality, despite progress in medical care[1-3]. In the last decade, many papers on the use of new urinary and serum biomarkers for AKI were published, mostly concluding that these biomarkers will lead to a new era with earlier diagnosis, better prognostication of outcome in terms of need for renal replacement and/or mortality, and finally better survival.[4] Nevertheless, there remains a gap between the fascinating findings at the basic science level and the clinical application of this knowledge. Objective evaluation of the available literature shows a rather disappointing picture.[5] Before we throw out the baby with the bathwater, we should evaluate what we reasonably can expect from biomarkers, what they provide today, and why their performance is currently suboptimal.

3.3 Methods

To identify relevant studies, we searched MEDLINE (through OvidSP) from inception to April 2012, using medical subject headings and textwords for AKI and biomarkers. The full search strategy is outlined in item S1, provided as online supplementary material. To locate studies not indexed in Medline, we checked by hand the bibliography of relevant publications. We included both cohort and case-control studies evaluating the potential of biomarkers to

detect AKI early, or to predict the need for renal replacement therapy in both adults and children. We excluded studies published as abstracts only and restricted to those published in English. Papers were excluded as "invalid intervention" when they did not analyse AKI, or where not performed in humans, and as "inappropriate design" when they did not investigate the differential diagnosis AKI vs non AKI. Mostly, the latter studies just reported means/medians in subgroups rather than discriminatory values on the individual patient level, or insufficient data were present to calculate these.We allowed for any definition of AKI. Biomarkers included in the search strategy were neutrophil gelatinase associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 18 (IL-18), Cystatin C, N-acetyl-glucosaminidase (NAG), liver fatty acid binding protein (LFABP) and the glutathione transferases (GST), measured either in plasma, serum or urine.

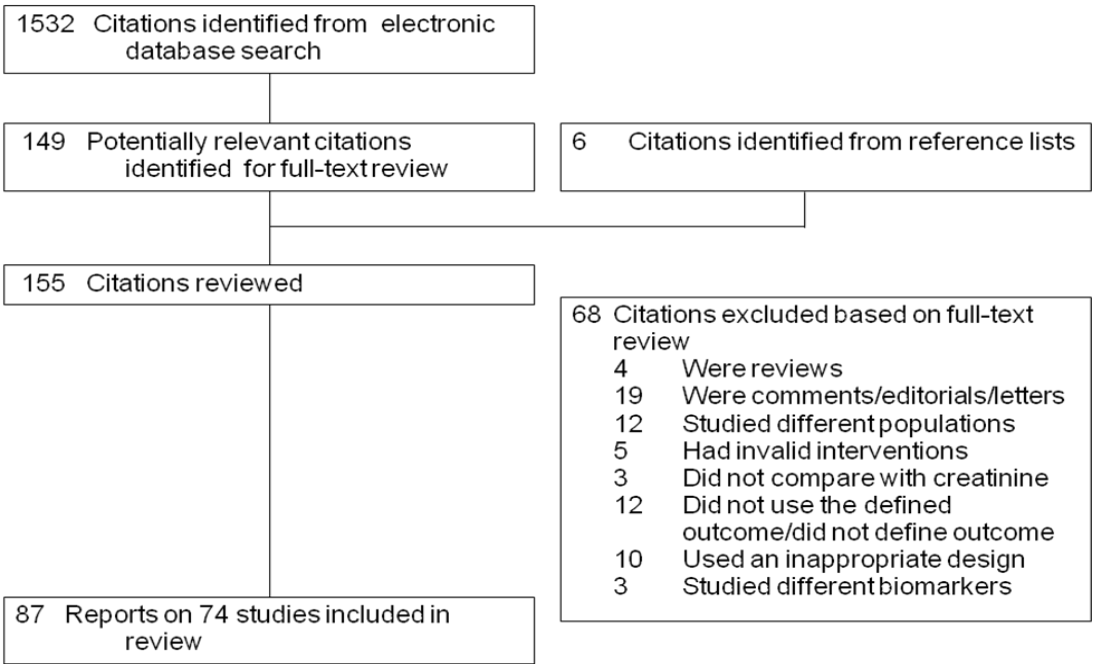
Two authors (JVM, WVB) independently screened all titles and abstracts and assessed all selected full papers for eligibility. Any disagreements were resolved by face to face discussion. Using a structured data extraction template, we collected details on biomarker(s) studied, outcome, AKI definition, number of patients/events and diagnostic test characteristics. Results are reported in overview tables 2 to 6. When not available, Positive Predictive Value (PPV) and Negatieve Predictive Value (NPV) were calculated if enough information was provided and if the study design allowed this.

Because of the heterogeneity of studies in different clinical settings, a meta-analysis or pooling of the data was deemed to be methodologically inappropriate.

3.4 Results

1532 potentially relevant papers were identified by the search strategy. Based on title and abstract, 149 papers were selected for full paper review. After checking the bibliography of relevant papers, 6 additional publications were selected for full paper review. 87 papers reporting on 74 studies, were included in the review. 68 papers were excluded for reasons outlined in figure 1.

Figure 1: Flowchart of retrieval and selection process of papers included in the final review.



All biomarkers and their abbreviations as used further in the text, are summarized in table 1.

Table 1: Biomarkers considered in this review

Acronym/abbreviation	Legend	Main source
AP	alcaline phosphatase	Liver, bone, intestine, placenta, brush border proximal convoluted tubules
α_1MG	alpha 1 microglobulin	Liver. Reabsorption by renal proximal tubular cells
$\alpha_1$acidGP	alpha 1 acid glycoprotein	Liver. Reabsorption by renal proximal tubular cells
AST	Aspartate aminotransferase	Liver, heart, muscle, brain, red blood cells, kidney
B_2MG	beta 2 microglobulin	All nucleated cells. Reabsorption by renal proximal tubular cells
Cystatin C	Cystatin C	All nucleated cells. Reabsorption by renal proximal tubular cells
FENA	Fractional Excretion of Sodium	
GGTP	Gamma glutamyl transpeptidase	All cells except myocytes. Mainly liver and kidney (brush border proximal convoluted tubules and loop of Henle)
GST-α	Alpha glutathione S transferase	Expressed in almost all tissues. Kidney: Proximal tubular cells (cytoplasmatic)
GST-π	Pi glutathione S transferase	Expressed in almost all tissues. Kidney: Distal tubular cells (cytoplasmatic)

Acronym/abbreviation	Legend	Main source
HGF	Hepatocyte Growth Factor	Mesenchymal cells
ICAM-1	Intracellular Adhesion Molecule 1	Endothelial cells, leukocytes
IL-6	Interleukin 6	T lymphocytes, macrophages, endothelial cells, monocytes
IL-8	Interleukin 8	Monocytes, macrophages, epithelial cells, endothelial cells
IL-10	Interleukin 10	Monocytes, lymphocytes, macrophages
IL-18	Interleukin 18	Monocytes, dendritic cells, macrophages and epithelial cells
KIM-1	Kidney Injury Molecule 1	Kidney: Proximal tubular cells
LDH	Lactate dehydrogenase	Heart, liver, red blood cells, muscle, brain, lung. Kidney: proximal tubular cells (cytoplasmatic)
LFABP	Liver type Fatty Acid Binding Protein	Hepatocytes, Kidney: proximal tubular cells
NGAL	Neutrophil Gelatinase Associated Lipocalin	Leucocytes, loop of Henle and collecting ducts
NAG	N Acetyl beta Glucosaminidase	Several tissues (Liver, brain, spleen,…). Kidney: Proximal tubular cells (lysosomal)
PAI-1	Plasminogen Activator Inhibitor 1	Endothelium
PCX	Podocalyxin	Podocytes
RBP	Retinol Binding Protein	Liver. Reabsorption by renal proximal tubula cells
sTNFR-I	Soluble Tumor Necrosis Factor Receptor I	Most cells and tissues (cytotoxic, apoptotic and proinflammatory effects)
sTNFR-II	Soluble Tumor Necrosis Factor Receptor II	Most cells and tissues (proliferative and anti-apoptotic effects)
TNFα	Tumor Necrosis Factor alpha	Macrophages, lymfoïd cells, renal parenchymal cells
11k-TXB_2	11-keto-Thromboxane B_2	Platelets
vWF	Von Willebrand Factor	Endothelium, megakaryocytes, subendothelial connective tissue

Results of the data extraction of the 87 selected papers are presented in tables 2 to 6. We organised papers according to 5 different clinical settings (pediatrics, cardiac surgery, emergency department, critically ill patients at ICU, and Contrast Induced Nephropathy(CIN)).

In the pediatric setting, 16 papers reporting on 11 studies were discussed. Eleven papers reporting on 7 studies were conducted post cardiac surgery[6-16], 1 studied CIN[17], 1 was conducted in the emergency department setting[18] and 3 papers reporting on 2 studies were conducted in critically ill children. Only 1 study in pediatric setting[6] explored the use of

biomarkers for early prediction of need for RRT (196 patients, 4 events, AUCRoC 0.86, PPV and NPV not available). All other studies considered the predictive value for AKI, which was defined according to 7 different definitions. The number of patients varied from 23 to 374, and the number of events varied from 6 to121. AURoC's for prediction of AKI varied from 0.44 to 1.00. PPV's and NPV's ranged from 27-100% and from 10-100%.

In adult cardiac surgery, 26 papers reporting on 22 studies were included. Twenty-seven different definitions for AKI were used. Patient number varied from 30-1219 and event number varied from 1-85. The AURoC's varied from 0.27-0.98. PPV's and NPV's ranged from 4-100% and from 61-100% respectively. One study[19] reported on the use of biomarkers for prediction of need for RRT (100 patients, 4 events, AURoC 0.83, PPV 100%, NPV 99%), although another study[20] also defined AKI as a 25% increase of Screa *or* RRT need with AURoC's between 0.54-0.73 and PPV 64-72%, NPV 67-73%.

In the emergency department setting, 4 studies were included[21-24]. Patient number varied from 616-1635 and event number from 24-130. Three different definitions of AKI were used. AURoC's varied from 0.59-0.95. PPV's and NPV's varied from 4-90% and from 84-99.5%.

In critically ill patients, 33 papers reporting on 29 studies of which 1 was a meta-analysis, were included. Twenty-one different definitions were used to define AKI. Patient number varied from 26-1345, event number varied from 4-209. AURoC's for prediction of AKI varied from 0.35-0.99 and PPV's and NPV ranged from 1-100% and from 50-100%. Fifteen papers reported on the adverse clinical outcomes of AKI (Failure of Recovery, RRT or the composite outcome of RRT and mortality). AURoC's for prediction of adverse clinical outcomes varied from 0.51-0.92 (PPV 5-75% and NPV 86-99.5%).

Eight studies exploring the use of biomarkers in CIN were included. Seven different definitions of CIN were used. Patient number ranged from 30-410 and event number from 2-34. AURoC's varied from 0.73-0.93. PPV's and NPV's ranged from 20-68% and from 96-100%.

3.5 Discussion

This paper evaluated the state of the art on the diagnostic usefulness of biomarkers in AKI. Most striking was the wide array of definitions used for AKI. With regard to the diagnostic usefulness of biomarkers, a variety of results ranging from overtly negative to very optimistic was found.

There are different explanations for this wide range of results. First, widely different clinical settings are investigated, from pediatric post cardiac bypass, where timing and amount of renal impact are exactly known, to patients with septic shock, where timing of renal insult is unknown. Overall, diagnostic performance of biomarkers appears to be better in situations with a known timing and etiology of renal injury. Second, most of these markers are not only associated with kidney damage, but also with the underlying conditions causing the AKI, such as sepsis, diabetic nephropathy , systemic lupus erythematosus and hemolytic uremic syndrome.[25-28] Some of these markers are also increased in chronic kidney disease[29;30], blurring the differential diagnosis between CKD and AKI. The reference baseline creatinine of a given patient is not always available, so in these patients, it is unclear whether an increased creatinine is due to AKI or chronic kidney disease (CKD). The behavior of most biomarkers in patients with CKD is however largely unknown. Usefulness of biomarkers

should thus be addressed differently for different clinical settings, as it is apparent that performance is strongly dependent on the underlying circumstances. As such, results in one setting cannot be generalised.

Third, one should be cautious for some statistical pitfalls when translating studies to clinical practice. Most studies report higher values for the biomarker in patient with AKI, but with substantial overlap between AKI and non AKI patients, hampering discrimination in the single case. Area under receiver operating characteristic (AURoC) curves are mostly used to circumvent this problem; however, the use of this instrument is troublesome in conditions with rather low prevalence, so also positive and negative predictive value should be provided, to allow good judgement on the accuracy of the biomarker. The use of odds ratios should be avoided in cases with low prevalence. In a study on performance of uNGAL for example, an impressive 16.4 higher odd's ratio to need RRT was reported; however, still only 2.5% of patients in this NGAL+/Screa- group end up on RRT, implicating that 97.5% did not. One should be cautious that, based on the high odds ratio, people become tempted to use NGAL cut-off levels to decide on start of RRT, as this would lead to unnecessary RRT in 97.5% of cases. [31-33]

Fourth, many studies use circular reasoning to define AKI by new markers. They start from the premise that an increase in the biomarker without a rise in Screa indicates AKI[34;35], thus increasing sensitivity, but at the expense of specificity and PPV. This confusion is due to the lack of a gold standard to diagnose AKI, and the difficulty to make a distinction between intrinsic renal damage (histological AKI) and physiologic decrease in renal perfusion (functional AKI) in the clinical setting. Undoubtedly, there is also a gray zone, where functional and histological AKI co-exist in a varying mix. Recent work by Nejat et al indicated that even in cases of transient AKI, markers of tubular damage can be present, demonstrating that "functional" and "intrinsic" AKI are probably gradations of an evolving spectrum of AKI.[36] Urinary markers will probably be more sensitive for true histological damage, whereas serum levels of markers are probably more sensitive for changes in clearance. In research conditions, e.g. to evaluate potential nephrotoxicity, use of high sensitivity markers is excellent. However, in clinical practice, we need to define what degree of subclinical damage as detected by biomarkers we will accept. Therefore, we need studies linking acute tubular damage to long term outcomes. Several studies make the link between AKI, even when apparently fully recovered, and progressive chronic kidney disease, but in all these, the classic definition of AKI, based on creatinine, was used, and to our knowledge, these data are lacking for biomarkers. [37;38]

Lastly, some studies advertise the use of biomarkers in situations where the outcome already seems predictable using standard parameters, such as clinical appraisal and oliguria.

In the study of Koyner et al, 7 patients needed RRT, 5 of them within 27 hours after cardiac surgery, making it plausible that RRT need in these patients could have been predicted by bedside clinical appraisal.[20] Also, the value of biomarkers to predict need for RRT should be evaluated compared to other available standards, such as clinical appreciation, time on cardiopulmonary bypass, or urinary output.[39] Singer et al used uNGAL to separate intrinsic from pre-renal AKI[40], but 32 out of 104 patients were esteemed "unclassifiable", and were thus not incorporated in the evaluation of the diagnostic accuracy of uNGAL. This means that only patients in whom the distinction between prerenal azotemia and AKI could already be established based on clinical grounds were included. From the provided graphs, it is clear

that the biomarker in these "unclassifiable" cases also was “intermediate”, and thus not discriminative.

Not taking into account all these reflections can lead to erroneous conclusions on the value of biomarkers, resulting in incorrect diagnoses, and thus incorrect therapeutic interventions.

It is clear from our survey that, whereas RIFLE and AKIN form a giant leap forward, the definition of AKI is still problematic, and that both RIFLE as AKIN are applied differently in every individual study in clinical practice, resulting in differences in classification.

The studies of Mishra et al and Bennett et al showed excellent performance for NGAL under ideal circumstances: children without comorbidities after cardiopulmonary bypass (CPB).[6;12] When more heterogenic populations were studied such as critically ill patients, results became more ambiguous.[41-43] Septic pediatric patients have higher uNGAL and IL-18 levels than non septic patients, indicating that the association between uNGAL and AKI in septic patients might more reflect the association between severity of disease and AKI rather than true renal damage.

Reported results on biomarkers in cardiac surgery are disappointing and conflicting, with AURoC’s for uNGAL comparable to the predictive power of clinical parameters such as duration of cardiopulmonary bypass and aortic cross–clamp time.[44-46] KIM-1 has been reported to have the best AURoC for the prediction of AKI. However, the low PPV showed that three out of four patients are being mislabeled as having AKI.[47] Plasma NGAL and IL-18 also performed poorly.[48;49] Serum cystatin C performed reasonably when AKI was defined as an increase in Screa of 50% , but poorly when based on RIFLE. More importantly, also Screa distinguished accurately between the AKI and non- AKI group within 24hs after arrival at ICU.[50] In subjects with a baseline eGFR < 60 ml/min/1.73m2, uNGAL was not able to predict the development of evolving AKI.[51] The discriminatory ability of uNGAL was less with decreasing RIFLE class.[19] The major challenge in the emergency setting, is to distinguish fluid responsive from non-fluid responsive AKI, and AKI from CKD. As we do not have an established clinical definition of "pre-renal" AKI, discrimination of this condition always remains somewhat subjective.

In a study with 635 patients, 411 had normal kidney function, 80 had prerenal azotemia, 30 AKI, and 106 non-progressive CKD.[21] This implicates that a patient with increased Screa at the emergency department has a six-fold higher odds to have either CKD or pre-renal kidney dysfunction than to have AKI. Despite the significant difference in median uNGAL levels between the different groups, there was a substantial overlap. Most of the patients with AKI had already very high Screa levels at presentation (mean 5.6 mg/dl, standard deviation 5.5 mg/dl). In patients presenting at the emergency department with suspected sepsis, median NGAL levels were higher in patients with vs. without AKI, but with substantial overlap, making discrimination per individual patient impossible.[23] In addition, patients who died without AKI had pNGAL levels comparable to the range of the AKI group, again indicating that the discriminative power of pNGAL for AKI is more related to the fact that it is a marker of severity of disease than of kidney injury.

In a prospective cohort study of 616 patients admitted to a tertiary care emergency department, both sCysC and Screa but not uCysC, differentiated between AKI and non-AKI. Authors state that sCysC distinguishes between AKI and prerenal azotemia. Neither biomarker discriminated between AKI and CKD.[24]

The application of biomarkers is most cumbersome in critically ill patients. As in this setting the timing of the renal insult is unknown, multiple samplings will have to be taken, which all have the potential of being false positive, and may lead to logistical and cost-effectiveness problems. The pathophysiology of AKI in sepsis patients is complex and not merely due to ischemia/reperfusion injury, but also to inflammatory processes.[52] It is likely that the levels of many biomarkers, e.g. IL-18, or NGAL, will be influenced by these processes, irrespective of AKI.[32] Plasma markers are thus in these patients more an indication of severity of disease than of true kidney damage.[53 54 33;55] Finding a biomarker or a panel of biomarkers that takes into account these different underlying pathological processes while still discriminating AKI is challenging. In most studies, there was no attempt to evaluate the discriminative power of the biomarker on top of other parameters, such as severity of sepsis, or urinary output. Although in a large cohort, uNGAL remained an independent predictor for AKI in a multivariate logistic regression model, the addition of uNGAL did not significantly improve discriminative power of the model. Overall, the clinical model performed better than uNGAL.[56] In ICU patients, uNGAL and pNGAL did perform worse than admission eGFR , and adding pNGAL and uNGAL to a multivariate model with eGFR only improved reclassification for AKI insignificantly.[57] Adding sCysC to a clinical model did not improve the diagnostic performance of the clinical model.[58] Urinary CysC predicted AKI, with a model including uCysC, uCrea, age, hypotension and APACHE II subcategory scores, in 444 critically ill patients, but when excluding patients with overt AKI on admission based on clinical grounds and Screa , the AURoC was only 0.54.[59] The predictive performance of urinary IL-18 in critically ill patients was poor[60], and uLFABP did not perform well to diagnose established AKI[61].

Some authors showed a better diagnostic performance of biomarkers when stratifying patients according to presence of pre-existing CKD. [60;62]

Another strategy to optimize the performance of biomarkers in ICU patients was to use a marker pattern (20 urinary peptides) rather than individual biomarkers.[63] However, a panel of 20 peptides measured by capillary electrophoresis-mass spectrometry, with high cost and long turn-around time, might not be realistic in clinical conditions.

Also in CIN, the use of biomarkers is prone to problems. The risk for CIN increases in patients with comorbid conditions, such as diabetes or older age, and in patients with pre-existing kidney disease, all of which influence on themselves biomarker levels. In patients without pre-existing CKD, the incidence of CIN is very low. In children, plasma and serum uNGAL were reported to be better predictors for CIN than Screa.[17] Diagnosis of AKI was made using NGAL and IL-18 in adults undergoing elective coronarography, PTCA or angioplasty, up to 24h before diagnosis by Screa, but this was not confirmed in another study.[64;65]

Interestingly NGAL is known to be increased in atherosclerotic plaques and might be released from these plaques during PCI without being related to kidney injury.[66] It can also be questioned whether CIN in patients with arteriography versus simple intravenous contrast administration, is the same condition, as the former can also be induced by cholesterol embolism.

3.6 Conclusion

Whereas biomarkers can increase our understanding of the pathophysiology of AKI, it appears that the promising results with new biomarkers for early detection and differential diagnosis of AKI in clinical practice can only be confirmed in the setting of children without comorbidities and with a well defined timing of renal injury. Results are far less robust when we search for validation in adult heterogenic populations, including patients with comorbid conditions such as diabetes mellitus, vascular disease and chronic kidney disease. Whereas AURoC values sometimes look impressive, applying levels of urinary or serum biomarkers for discrimination in individual patients is hampered by wide overlap between groups, which might result in many false positives. Biomarkers reflect a general degree of severity of disease, rather than being specific for kidney injury. Before biomarkers can be advocated to diagnose AKI, further research on the implications of "subclinical" AKI, i.e. diagnosed by the biomarker but not by an increase of creatinine or decrease of diuresis, should be performed

Table 2: Pediatric setting

Author	PY	Biomarker	U/S/P	Outcome	AKI def/Outcome def	Patients/Events	AURoC	PPV (%)	NPV (%)
Mishra[12]	2005	NGAL	U	AKI	Screa * 1.5	71/20	0.99	95	100
			S				0.91	82	89
Hirsch[17]	2007	NGAL	U	CIN	Screa * 1.5	91/11	0.92	100	96
			P				0.91	80	96
Zappitelli[43]	2007	NGAL	U	AKI	pRIFLE crea	140/106	0.78	NA	NA
				Persistent AKI	AKI lasting > 48h	140/?	0.79	NA	NA
Dent[8]	2007	NGAL	P	AKI	Screa * 1.5	120/45	0.96	84	93
Bennet[6]	2008	NGAL	U	AKI	Screa * 1.5	196/99	0.95	89	83
				RRT		196/4	0.86	NA	NA
Wheeler[42]	2008	NGAL	S	AKI	BUN>100mg/dl, Screa>2mg/dl in absence of CKD or RRT	143/22	0.68	39	94
Krawczeski[10]	2011	NGAL	U	AKI	Neonates:↑Screa >0.3mg/dl	374/112	0.95(neonates)	80	100
							0.92 (others)	73	93
			P		Children: Screa *1.5 within 48h after CPB		0.95 (neonates)	78	96
							0.94 (others)	77	95
Portilla[14]	2008	NGAL	U	AKI	Screa * 1.5	40/21	1.00	100	100
Du[18]	2011	NGAL	U	AKI	pRIFLE crea	252/18	0.66-0.67	NA	NA
Parikh[13]	2011	NGAL	U	AKI	Progression of established AKI	311/53	0.71	30	93
			P				0.56	20	86
Parikh[13]	2011	IL-18	U	AKI	Progression of established AKI	311/53	0.72	30	92
Parikh[16]	2006	IL-18	U	AKI	Screa * 1.5	55/20	0.75	65	76

Author	PY	Biomarker	U/S/P	Outcome	AKI def/Outcome def	Patients/Events	AURoC	PPV (%)	NPV (%)
Du[18]	2011	IL-18	U	AKI	pRIFLE crea	252/6	0.44-0.54	NA	NA
		IL-18	U	RIFLE-I			0.48-0.64	NA	NA
Washburn[41]	2008	IL-18	U	AKI	pRIFLE crea	137/103	0.54	27	85
Portilla[14]	2008	LFABP	U	AKI	Screa * 1.5	40/21	0.81	71	68
Dennen[7]	2010	IL-6	U	AKI	Screa*1.5	23/10	NA	60	10
Liu[11]	2009	IL-6	S	AKI	Screa *1.5 within 3 days	39/18	0.76	69	87
		IL-8	S				0.74	52	78
Zappitelli[15]	2011	Clinical Model(CM)	S	AKI	Screa*1.5	288/121	0.71	NA	NA
		Cystatin C + CM					0.81	NA	NA
		Screa + CM					0.83	NA	NA
Krawczeski[9]	2010	Cystatin C	S	AKI	Screa *1.5 within 48h	374/119	0.81	70	87
Du[18]	2011	KIM-1	U	AKI	pRIFLE crea	252/18	0.61	NA	NA
		β2-MG	U				0.59	NA	NA
		KIM-1	U	RIFLE-I		252/6	0.73	NA	NA
		β2-MG	U				0.80	NA	NA

Abbreviations: PY: Publication Year, U/S/P: Urine/Serum/Plasma, AKI def: definition of Acute Kidney Injury, AURoC: Area Under the Receiver Operating Characteristic Curve, PPV: Positive Predictive Value, NPV: Negative Predictive Value, NA: Not Available or Not Applicable, NGAL: Neutrophil Gelatinase-Associated Lipocalin, IL-18: Interleukin 18, LFABP: Liver Fatty Acid Binding Protein, IL-6: Interleukin 6, IL-8: Interleukin 8, KIM-1: Kidney Injury Molecule 1, β2MG: β2-microglobulin, Screa: Serum Creatinine, BUN: Blood Urea Nitrogen,AKI: Acute Kidney Injury, CKD: Chronic Kidney Disease, CIN: Contrast Induced Nephropathy, RRT: Renal Replacement Therapy, pRIFLE: pediatric modified Risk Injury Failure Loss of Kidney Function and End stage renal disease classification, pRIFLE crea: pRIFLE criteria based on the creatinine criterium (omitting the diuresis criterion), CPB: Cardiopulmonary bypass time

Table 3: Cardiac Surgery

Author	PY	Biomarker	U/S/P	Outcome	AKI def/Outcome def	Patients/Events	AURoC	PPV (%)	NPV (%)
Parikh[67]	2011	NGAL	U	AKI	Doubling of Screa	1219/60	0.67	NA	NA
			P				0.70	NA	NA
		Screa immediate postop	P				0.72	NA	NA
Xin[68]	2008	NGAL	U	AKI	AKIN both	33/9	0.88	58	91
Wagener[69]	2007	NGAL	U	AD	Scra *1.5	81/6	0.8	4	9
Wagener[46]	2008	NGAL	U	AKI	AKIN crea	426/85	0.61	31	84
Haase-Fielitz[50]	2009	NGAL	P	AKI	Screa *1.5 within 5 days postoperatively	100/23 eGFR > 60 ml/min/1.73m^2	0.80/0.87 (ICU arr/24h post)	52/53	93/97
		NGAL	P	AKI	RIFLE		0.80/0.87	69/57	94/95
		NGAL	P	AKI RIFLE		100/5	0.73/0.70	73/69	69/61
		NGAL	P	RRT+M			0.95	57	99
		NGAL	P	RRT+M		eGFR > 60 ml/min/1.73m2	NA	NA	NA
Haase-Fielitz[19]	2009	NGAL	P	AKI	-Screa * 1.25 within 48h	100/36	0.66	58	78
					-Screa * 1.25 within 72h	100/38	0.64	55	74
					-Screa * 1.25 within 120h	100/39	0.67	55	76
					-Screa * 1.25 within 168h	100/40	0.64	57	72
					-↑ Screa 0.3mg/dl or *1.5 48h	100/32	0.66	51	80
					-Screa * 1.25 or RRT within 72h	100/42	0.68	66	75
					-Screa * 1.5 within 48h	100/20	0.78	44	92
					-Screa * 1.5 within 72h	100/21	0.79	46	92
					-Screa * 1.5 within 120h	100/23	0.80	51	92
					-Screa * 1.5 within 168h	100/23	0.80	51	92

				RIFLE R	RIFLE both	100/31	0.72	52	84
				RIFLE I		100/13	0.79	33	97
				RIFLE F		100/6	0.80	100	98
				AKIN I	AKIN both	100/29	0.75	53	87
				AKIN II		100/11	0.78	33	97
				AKIN III		100/6	0.81	100	98
				RRT		100/4	0.83	100	99
Tuladhar[70]	2009	NGAL	U	AKI	↑ Screa 0.5mg/dl within 48h	50/9	0.96	47	97
			P				0.85	33	93
Perry[49]	2010	NGAL	P	AKI	Screa *1.5 within 4 days	879/75	0.64	16	93
Prabhu[71]	2010	NGAL	P	AKI	RIFLE crea	30/8	0.98	79	100
McIllroy[51]	2010	NGAL	U	AKI	AKIN crea	426/85			
					-eGFR<30	21/9	0.34	44	60
					-eGFR 30-60	101/20	0.51	22	86
					-eGFR 60-90	142/35	0.55	29	85
					-eGFR 90-120	109/13	0.88	40	99
					-eGFR > 120	53/8	0.27	4	74
Koyner[20]	2008	NGAL	P	AKI	Screa *1.25 or RRT need within 72h	72/34	0.54	NA	NA
		NGAL	U				0.69	64	67
Haase[72]	2009	NGAL	P	AKI	AKIN both	100/46	0.77	71	77
		NGAL+CysC	S				0.81	78	75
Koyner[73]	2012	NGAL	U	AKI	AKI progression	380/45	NA	NA	NA
			P						
Koyner[45]	2010	NGAL	U	AKI stage 1	AKIN	123/36	0.72	NA	NA
		NGAL	U	AKI	-eGFR>60	74/27	0.81	NA	NA
		NGAL	U	AKI	-eGFR<60	49/19	0.58	NA	NA
		NGAL	U	AKI stage 3		123/9	0.88	NA	NA
		NGAL	U	AKI stage 3	-eGFR>60	74/4	0.97	NA	NA
		NGAL	U	AKI stage 3	-eGFR<60	49/5	0.73	NA	NA
Heise[74]	2011	NGAL	U	AKI	AKIN both	47/38	0.77	94	50

Author	PY	Biomarker	U/S/P	Outcome	AKI def/Outcome def	Patients/Events	AURoC	PPV (%)	NPV (%)
Han[44]	2009	NGAL	U	AKI	↑ Screa 0.3mg/dl or 2 to 3 fold increase in Screa within 72h	90/36	0.59/0.65 (imm/3h postop)	47/57	69/77
		NGAL	U	Early AKI	↑ Screa 0.3mg/dl within 24h	90/16	0.51/0.58	NA	NA
		NGAL	U	Late AKI		90/20	0.66/0.71	NA	NA
Liangos[47]	2009	NGAL	U	AKI	Screa *1.5 within 72h	103/13	0.50	15	90
Koyner[20]	2008	Cystatin C	P	AKI	Screa *1.25 or RRT need within 72h	72/34	0.62	NA	NA
		Cystatin C	U				0.73	72	73
Haase-Fielitz[50]	2009	Cystatin C	S	AKI	Screa *1.5 within 5 days postoperatively	100/23	0.83/0.84	62/43	93/96
		Creatinine	S				0.68/0.86	47/56	85/93
		Ureum	S				0.60/0.79	32/53	90/88
		Cystatin C	S	AKI		eGFR > 60 ml/min/1.73m^2	0.78/0.84	64/53	93/96
		Creatinine	S				0.69/0.86	54/49	86/97
		Ureum	S				0.61/0.79	35/36	89/100
		Cystatin C	S	AKI RIFLE	RIFLE	100/5	0.75/0.73	75/69	69/71
		Creatinine	S				0.58/0.73	56/75	53/65
		Ureum	S				0.55/0.76	54/76	55/62
		Cystatin C	S	RRT+M			0.99	36	100
		Creatinine	S				NA	NA	NA
		Ureum	S				NA	NA	NA
		Cystatin C	S	RRT+M		eGFR > 60 ml/min/1.73m^2	NA	NA	NA
		Creatinine	S				NA	NA	NA
		Ureum	S				NA	NA	NA
Haase[72]	2009	Cystatin C	S	AKI	AKIN both	100/46	0.76	65	75
Ristikankare[75]	2010	Cystatin C	S	AKI	RIFLE both POD1	110/62	0.71/0.77 (POD1/POD2)	NA	NA
		Creatinine	P		RIFLE crea POD2		0.66/0.74 (POD1/POD2)	NA	NA
Wald[76]	2010	Cystatin C	P	AKI	Screa *1,5 or ↑ 0,3 mg/dl within 72h	150/47	0.68	NA	NA

Author	PY	Biomarker	U/S/P	Outcome	AKI def/Outcome def	Patients/Events	AURoC	PPV (%)	NPV (%)
Heise[74]	2011	Cystatin C	U	AKI	AKIN both	47/38	0.59	NA	NA
		α_1MG	U				0.61	NA	NA
Koyner[45]	2010	Cystatin C	U	AKI stage 1	AKIN	123/36	0.72	NA	NA
		Cystatin C	U	AKI	-eGFR>60	74/27	0.70	NA	NA
		Cystatin C	U	AKI	-eGFR<60	49/19	0.70	NA	NA
		Cystatin C	U	AKI stage 3		123/9	0.85	NA	NA
		Cystatin C	U	AKI stage 3	-eGFR>60	74/4	0.93	NA	NA
		Cystatin C	U	AKI stage 3	-eGFR<60	49/5	0.81	NA	NA
Liangos[47]	2009	CystatinC	U	AKI	Screa *1.5 within 72h	103/13	0.50	31	91
Xin[68]	2008	IL-18	U	AKI	AKIN both	33/9	0.89	78	92
Liangos[47]	2009	IL-18	U	AKI	Screa *1.5 within 72h	103/13	0.66	25	95
Liang[77]	2010	IL-18	U	AKI	RIFLE crea	122/30	0.62	27	79
				Progr AKI	RIFLE R → I or RIFLE ≥ I from start	Progr AKI: 122/11	0.91	17	100
Haase[48]	2008	IL-18	U	RIFLE ≥ R	RIFLE within 120h. Urine output criterion	100/50	0.61/0.57 (ICU arr/24h post)	NA	NA
					only during first 24h	100/19	0.52/0.58	NA	NA
				RIFLE ≥ I	-Screa * 1.25 within 24h	100/?	0.58/0.56	NA	NA
					-Screa * 1.25 within 48h	100/?	0.59/0.56	NA	NA
				AKI	-Screa * 1.25 within 72h	100/?	0.60/0.58	NA	NA
					-Screa * 1.25 within 120h	100/?	0.60/0.59	NA	NA
					-Screa * 1.5 within 24h	100/?	0.56/0.57	NA	NA
					-Screa * 1.5 within 48h	100/20	0.53/0.55	NA	NA
					-Screa * 1.5 within 72h	100/?	0.58/0.55	NA	NA
					-Screa * 1.5 within 120h	100/?	0.52/0.56	NA	NA
				AKI	AKIN ≥ Stage1	100/32	0.48/0.59	NA	NA
				Sustained	-Screa * 1.25 within 48h	100/?	0.58/0.56	NA	NA
				AKI (At least	-Screa * 1.25 within 120h	100/?	0.59/0.56	NA	NA
				two	-Screa * 1.5 within 48h	100/?	0.56/0.57	NA	NA
				consecutively	-Screa * 1.5 within 120h	100/?	0.51/0.57	NA	NA
				increased	-RIFLE ≥ R	100/50	0.61/0.57	NA	NA
				Screa levels)	-RIFLE ≥ I	100/19	0.52/0.58	NA	NA

Author	PY	Biomarker	U/S/P	Outcome	AKI def/Outcome def	Patients/Events	AURoC	PPV (%)	NPV (%)
Han[44]	2009	KIM-1	U	AKI	↑ Screa 0.3mg/dl or 2 to 3 fold increase in Screa within 72h	90/36	0.68/0.65	61/72	71/68
		NAG	U				0.61/0.63	50/54	75/69
		Panel of 3	U				0.75/0.78	NA	NA
		KIM-1	U	Early AKI	↑ Screa 0.3mg/dl within 24h	90/16	0.79/0.73	NA	NA
		NAG	U				0.60/0.59	NA	NA
		Panel of 3	U				0.80/0.84	NA	NA
		KIM-1	U	Late AKI	↑ Screa 0.3mg/dl within 24-72h	90/20	0.61/0.60	NA	NA
		NAG	U				0.62/0.65	NA	NA
		Panel of 3	U				0.72/0.74	NA	NA
Liangos[47]	2009	KIM-1	U	AKI	Screa *1.5 within 72h	103/13	0.78	24	98
		KIM1/NAG/IL18					0.78	NA	NA
		KIM-1/CPB time					0.78	NA	NA
		KIM-1/CCF					0.88	NA	NA
Liang[77]	2010	KIM-1	U	AKI	RIFLE crea	122/30	0.88	57	96
				Progr AKI	RIFLE R → I or RIFLE ≥ I from start	Progr AKI: 122/11	0.70	19	97
		KIM-1+IL-18	U	Progr AKI			0.90	35	90
Koyner[45]	2010	KIM-1	U	AKI stage 1	AKIN	123/36	0.67	NA	NA
		KIM-1	U	AKI		74/27	0.68	NA	NA
		KIM-1	U	AKI	-eGFR>60	49/19	0.64	NA	NA
		KIM-1	U	AKI stage 3	-eGFR<60	123/9	0.82	NA	NA
		KIM-1	U	AKI stage 3	-eGFR>60	74/4	0.80	NA	NA
		KIM-1	U	AKI stage 3	-eGFR<60	49/5	0.82	NA	NA
Jörres[78]	1994	βNAG	U	Renal Injury	Screa > 1.3 mg/dl	36/12	NA	NA	NA
		α_1MG	U						
		Albumine	U						
		Transferrin	U						
		IgG	U						
		11k-TXB_2	U						
da Silva Magro[79]	2004	α GST	U	ARF	↓CrCl<75ml/min within 72h	41/20	< 0.80	NA	NA
		FENA	U				NA	NA	
		Creatinine	P				NA	NA	
		Urea	P				NA	NA	
		CrCl					NA	NA	

Author	PY	Biomarker	U/S/P	Outcome	AKI def/Outcome def	Patients/Events	AURoC	PPV (%)	NPV (%)
Eijkenboom[80]	2005	αGST	U	ARF	Screa *1.5	84/1	NA	NA	NA
		πGST	U						
Wagener[46]	2008	CPB time	U	AKI	AKIN crea	426/85	0.59	NA	NA
		AXT					0.59	NA	NA
Liangos[47]	2009	NAG	U	AKI	Screa *1.5 within 72h	103/13	0.62	26	91
		α_1MG					0.62	21	94
		CPB perf time					0.67	NA	NA
		CCF score					0.83	NA	NA
Liangos[81]	2009	IL-8	P	AKI	1. Screa *1,5 or ↑ 0,3 mg/dl	143/41	0.62	NA	NA
					2. Screa *1.5within 72h	143/18	0.72	NA	NA
Gueret[82]	2009	IL-6	P	ARD	Faint ARD: Screa *1.25 Moderate ARD: Screa *1.5 Severe ARD: Screa *2 within 48h	63/36	NA	NA	NA
Koyner[45]	2010	HGF	U	AKI stage 1	AKIN	123/36	0.53	NA	NA
		αGST	U				0.62	NA	NA
		πGST	U				0.60	NA	NA
		FENA	U				0.49	NA	NA
		FEUrea	U				0.59	NA	NA
		HGF	U	AKI	-eGFR>60	74/27	0.52	NA	NA
		αGST	U				0.66	NA	NA
		πGST	U				0.61	NA	NA
		FENA	U				0.50	NA	NA
		FEUrea	U				0.52	NA	NA
		HGF	U	AKI	-eGFR<60	49/19	0.54	NA	NA
		αGST	U				0.57	NA	NA
		πGST	U				0.56	NA	NA
		FENA	U				0.46	NA	NA
		FEUrea	U				0.51	NA	NA
		HGF	U	AKI stage 3		123/9	0.65	NA	NA
		αGST	U				0.68	NA	NA
		πGST	U				0.78	NA	NA
		FENA	U				0.65	NA	NA
		FEUrea	U				0.57	NA	NA

	HGF	U	AKI stage 3	-eGFR>60	74/4	0.73	NA	NA
	αGST	U				0.75	NA	NA
	πGST	U				0.93	NA	NA
	FENA	U				0.66	NA	NA
	FEUrea	U				0.60	NA	NA
	HGF	U	AKI stage 3	-eGFR<60	49/5	0.44	NA	NA
	αGST	U				0.61	NA	NA
	πGST	U				0.56	NA	NA
	FENA	U				0.63	NA	NA
	FEUrea	U				0.35	NA	NA

Abbreviations: Same as in table 2. Not defined in table 2: ARF: Acute Renal Failure, ARD: Acute Renal Dysfunction, Progr AKI: Progressive AKI, ICU: Intensive Care Unit, imm: immediately, ICU arr: ICU arrival, 24h post: 24h postoperatively, imm/3h: immediately/3 hours after surgery, POD: postoperative day, AKIN: Acute Kidney Injury Network, RIFLE crea: staging according to RIFLE based on the creatinine criterion (omitting the diuresis criterion), AKIN crea: staging according to AKIN based on the creatinine criterion (omitting the diuresis criterion); RIFLE/AKIN both: staging according to RIFLE or AKIN based on both criteria (creatinine and diuresis)CrCl: Creatinine Clearance, eGFR: estimated Glomerular Filtration Rate, αGST: α-glutathione-S-transferase, πGST: π-glutathione-S-transferase, α_1MG: α_1 microglobulin , AXT: aortic cross-clamp time, βNAG: N-acetyl-β-D-glucosaminidase, CysC: Cystatine C, CCF: Cleveland Clinic Foundation Score , CPB time: CPB perfusion time, IgG: Immunoglobulin G, HGF: Hepatocyte Growth Factor, 11k-TXB_2: 11-keto-thromboxane B_2, FENA: Fractional Excretion of Sodium, FEUrea: Fractional Excretion of Urea

Table 4: Emergency department

Author	PY	Biomarker	U/S/P	Outcome	AKI def/Outcome def	Patients/Events	AURoC	PPV (%)	NPV (%)
Nickolas[21]	2008	NGAL	U	AKI	RIFLE crea	635/30	0.95	90	99.5
Shapiro[23]	2010	NGAL	P	AKI	↑0,5mg/dl or RRT need within 72h	661/24	0.82	7	99
				≥RIFLE R	Screa * 1,5	661/27	NA	7	98
				≥RIFLE I	Screa * 2	661/15	NA	4	99
Nickolas[22]	2012	NGAL	U	Intrinsic AKI		1635/96	0.81	23	97
		T0 Creatinine>1.4	S				0.90	28	98
		T0 Creatinine>1.1	S				NA	17	99
		IL-18	U				0.64	14	94
		KIM-1	U				0.71	17	95
Nickolas[21]	2008	NAG	U	AKI	RIFLE crea	635/30	0.71	9	98
		α_1MG	U				0.89	17	99
		$\alpha_1$acidGP	U				0.83	10	99
		FENA	U				0.71	16	94
		Creatinine	S				0.92	35	99
Shapiro[23]	2010	Creatinine	P	≥RIFLE I	Screa * 1,5	661/27	NA	4	99
				AKI	Screa * 2	661/15	NA	3	99
Soto[24]	2010	Cystatin C	S	AKI	AKIN crea	616 AKI: 130 Prerenal azotemia: 159 Stable CKD: 15	0.87	48	94
			U				0.59	32	84
		Creatinine	S	AKI	AKIN crea		0.9	54	95
			U				0.62	NA	NA

Abbreviations: Same as in tables 2 and 3. Not defined in tables 2 and 3: $\alpha_1$acidGP: α_1 acid glycoprotein; T0: at admission

Table 5: Critically ill patients at ICU

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Ahlström[83]	2004	Cystatin C	S	ARF	RIFLE F both	202/54	0.89	NA	NA
Herget-Rosenthal[84]	2004	Cystatin C	U	RRT	*patients with non oliguric ATN*	73/26	0.92	75	95
Herget-Rosenthal[85]	2004	Cystatin C	S	ARF	RIFLE crea	85/44	Rday-2/-1: 0.82/0.97	92/95	66/83
							Iday-2/-1: 0.92/0.98	100/100	63/81
		Cystatin C	S	RRT		85/17	Fday-2/-1:0.97/0.99	100/100	76/93
							Rday-2/-1:0.69/0.75	45/76	86/93
Mazul-Sunko[86]	2004	Cystatin C	P	ARF	Screa ≥ 267 µmol/l or diuresis < 30ml/h in patients without CKD	29/10	NA	NA	NA
Hei[87]	2008	Cystatin	S	ARF	↑Screa to 132 µmol/l or ↑BUN to 18 mmol/l	60/10	NA	42	85

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Perianayagam[58]	2009	Cystatin C	S	RRT or M	*Inlcusion of patients with ARF defined as*BL ≤1.9mg/dl: ↑Screa 0.5 mg/ dl	200/84	0.65	NA	NA
		Clinical model + Cystatin C					0.83	NA	NA
		Clinical Model + Creatinine					0.83	NA	NA
		Clinical Model + Urea					0.84	NA	NA
		Clinical Model + Urine output					0.84	NA	NA
		Clinical model alone					0.82	NA	NA
Portal[88]	2010	Cystatin C	S	AKI	AKIN crea	80/30	0.78	NA	NA
		Cystatin C	S	Severe AKI	Severe AKI: ≥ stage 2	80/19	0.78	NA	NA
Nejat[89]	2010	Cystatin C	P	-AKI	AKIN crea	444/198	0.78	NA	NA
		Creatinine	S				0.87	NA	NA
		Cystatin C	P	-AKI 7d	AKIN crea within 7 days	319/73 (no AKI on entry)	0.65	NA	NA
		Creatinine	S				0.61	NA	NA
		Cystatin C	P	-AKI sust	Screa*1,5 within 7 days and ≥24h	319/19 (no AKI on entry)	0.80	NA	NA
		Creatinine	S				0.57	NA	NA
		Cystatin C	P	-RRT		319/? (no AKI on entry)	0.84	NA	NA
		Creatinine	S				0.77	NA	NA

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Nejat[59]	2010	Cystatin C	U	AKI 48h	AKIN crea	319/73 (no AKI on entry)	0.54	NA	NA
			U	AKI 48h		51/? (sepsis, no AKI on entry)	0.71	NA	NA
			P	AKI 48h		268/? (no sepsis, no AKI on entry)	Not predictive	NA	NA
			U	AKI 48h		268/? (no sepsis, no AKI on entry)	0.45	NA	NA
			P	AKI 48h			NA	NA	NA
Metzger[63]	2010	Cystatin C	S	AKI	AKIN both 2/16	20/9	0.67	NA	NA
Endre[62]	2011	Cystatin C	U	AKIN48	AKIN crea	381 (no AKI on entry)/82	0.55	NA	NA
		Cystatin C	U	RIFLE 24hrs	RIFLE crea, sustained for ≥24h	381/27	0.63	NA	NA
		Cystatin C	U	AKIN48	-eGFR < 60ml/min	69/20	0.64	NA	NA
					-eGFR 60-<90 ml/min	116/25	0.54	NA	NA
					-eGFR 90-<120 ml/min	128/26	0.58	NA	NA
					-eGFR > 120 ml/min	66/11	0.35	NA	NA
		Cystatin C	U	RRT		128/26	0.66	NA	NA

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Royakkers[90]	2011	Cystatin C	U	AKI	RIFLE both	151 No-AKI: 60 AKI at adm.: 56	day-2: 0.49 day-1: 0.46	NA NA	NA NA
			S	AKI	RIFLE both	AKI after adm.: 35	day-2: 0.72 day-1: 0.62	NA NA	NA NA
			U	RRT		151/14	0.61	NA	NA
			S				0.66	NA	NA
Niemann[91]	2009	NGAL	S	AKI	RIFLE crea	59/27	NA	NA	NA
						45 (Screa baseline < 1.5mg/dl)/24	0.79	NA	NA
Makris[92]	2009	NGAL	U	AKI	RIFLE both	31/11	0.98	91	95
		Creatinine	S				0.79	NA	NA
Aghel[93]	2010	NGAL	S	Worsening renal function	↑Screa≥0.3mg/dl within 5 days	91/35	0.70	54	86
		BUN	S				0.56	NA	NA
		eGFR					0.61	NA	NA
Bagshaw[55]	2010	NGAL	P	Worsening AKI/RRT	RIFLE	83 (sepsis:43)/20	0.71/0.78	NA	NA
			U			RRT:13	0.70/0.70	NA	NA
Märtensson[94]	2010	NGAL	U	AKI	RIFLE or AKIN both	45/18	0.86	100	84
			P				0.85	79	88
		NGAL	U			25(only septic shock)/18	0.86	100	58
			P				0.67	81	54

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Siew[56]	2009	NGAL	U	AKI 24h	AKIN crea	451/64	0.71	NA	NA
		Clinical					0.81	NA	NA
		Model					0.82	NA	NA
		NGAL+clinical model							
		NGAL	U	AKI 24h	AKIN crea,	275/18	0.77	NA	NA
		NGAL	U	AKI 48h	eGFR ≥ 75 ml/min	451/86	0.64	NA	NA
		NGAL	U	Sust AKI 24h	Persistent AKI 24h post detection	451/47	0.70	NA	NA
			U	Sust AKI 48h	Persistent AKI 24h post detection	451/52	0.66	NA	NA
Constantin[32]	2010	NGAL	P	AKI	RIFLE crea	88/52	0.93	97	80
						56/20 (no AKI on admission)	0.96	94	92
				RRT		88/7	0.79	21	98
Cruz[33]	2010	NGAL	P	AKI	RIFLE both within 5 daysAKI within 48h	301/133	O.67	26	91
				AKI 48h		301/?	0.78	24	97
				RRT		133/15	0.82	12	99
Yang[95]	2010	NGAL	U	Failure of recovery	Failure to return to baseline or RRT need *Inclusion of AKI patients according to RIFLE crea*	100/35	0.88	72	93

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Endre[62]	2011	NGAL	U	AKIN48	AKIN crea	381 (no AKI on entry)/82	0.55	NA	NA
		NGAL	U	RIFLE 24hrs	RIFLE crea,	381/27	0.68	NA	NA
		NGAL	U	AKIN48	sustained for ≥24h				
			U		-eGFR < 60ml/min	69/20	0.71	NA	NA
			U		-eGFR 60-<90 ml/min	116/25	0.53	NA	NA
			U		-eGFR 90-<120 ml/min	128/26	0.53	NA	NA
			U		-eGFR > 120 ml/min	66/11	0.44	NA	NA
		NGAL		RRT			0.78	NA	NA
Haase[31]	2011	NGAL	U	RRT		1345/29	NA	5	99.5
			P	RRT		977/30	NA	6	99
		Creatinine	S	RRT		1345/29	NA	6	99
De Geus[57]	2011	NGAL	P	RIFLE R	RIFLE crea	632/67	0.77	40	97
				RIFLE I		632/48	0.80	NA	NA
				RIFLE F		632/56	0.86	NA	NA
			U	RIFLE R		632/67	0.80	35	95
				RIFLE I		632/48	0.85	NA	NA
				RIFLE F		632/56	0.88	NA	NA
		eGFR		RIFLE R		632/67	0.84	NA	NA
				RIFLE I		632/48	0.87	NA	NA
				RIFLE F		632/56	0.92	NA	NA
		NGAL	P	RIFLE I/F	RIFLE crea, eGFR > 60 ml/min	632/104	0.75	NA	NA
			U	RIFLE I/F	RIFLE crea, eGFR > 60 ml/min	632/104	0.79	NA	NA
			S	RIFLE I/F	RIFLE crea, eGFR > 60	632/104	0.65	NA	NA

					ml/min				
		NGAL	P	RIFLE I/F	RIFLE crea, eGFR > 60 ml/min	632/104	0.67	NA	NA
		Creatinine		RIFLE F	RIFLE crea	632/56	0.95	NA	NA
		eGFR		RIFLE F	RIFLE crea	632/56	0.96	NA	NA
		Clinical Model		RIFLE F	RIFLE crea	632/56	0.94	NA	NA
		Clinical Model +NGAL	U	RIFLE F	RIFLE crea	632/56	0.95	NA	NA
		Clinical Model		RRT		632/28	0.89	NA	NA
		Clinical Model+NGAL	U	RRT			0.88	NA	NA
		NGAL	P	RRT			0.90	NA	NA
		Creatinine eGFR	S				0.91	NA	NA
Metzger[63]	2010	NGAL	U	AKI	AKIN crea	20/9	0.54	NA	NA
Siew[60]	2010	NGAL	U	AKI 24h	AKIN crea	451/64	0.71	NA	NA
		NGAL+IL-18	U				0.71	NA	NA
Portal[88]	2010	NGAL	P	AKI	AKIN crea	80/30	0.79	71	81
			U				0.76	NA	NA
		NGAL	P	Severe AKI	Severe AKI: ≥ stage 2	80/19	0.87	61	91
			U				0.84	NA	NA
		NGAL	S	RRT			0.84	NA	NA

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Endre[62]	2011	IL-18	U	AKIN48	AKIN crea	381 (no AKI on entry)/82	0.55	NA	NA
		IL-18	U	RIFLE 24hrs	RIFLE crea, sustained for ≥24h	381/27	0.72	NA	NA
		IL-18	U	AKIN48	-eGFR < 60ml/min	69/20	0.65	NA	NA
			U		-eGFR 60-<90 ml/min	116/25	0.48	NA	NA
		IL-18	U		-eGFR 90-<120 ml/min	128/26	0.57	NA	NA
			U		-eGFR > 120 ml/min	66/11	0.49	NA	NA
			U	RRT			0.70	NA	NA
Siew[60]	2010	IL-18	U	AKI 24h	AKIN crea	451/64	0.62	NA	NA
		IL-18	U	AKI 24h	AKIN crea, eGFR ≥ 75 ml/min	275/18	0.67	NA	NA
		IL-18	U	AKI 48h	AKIN crea	451/86	0.60	NA	NA
		IL-18	U	AKIN I	AKIN crea	451/61	0.59	NA	NA
		IL-18	U	AKIN II+III	AKIN crea	451/25	0.62	NA	NA
		IL-18	U	RRT		451/17	NA	NA	NA
Metzger[63]	2010	IL-18	U	AKI	AKIN crea	20/9	0.57	NA	NA
Parikh[96]	2005	IL-18	U	AKI	Screa * 1.5 within 6 days	138/52	24h before ↑Screa: 0.73	62	78
							48h before ↑Screa: 0.65	NA	NA

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Endre[62]	2011	KIM-1	U	AKIN48	AKIN crea	381 (no AKI on entry)/82	0.55	NA	NA
		KIM-1	U	RIFLE 24hrs	RIFLE crea, sustained for ≥24h	381/27	0.64	NA	NA
		KIM-1	U	AKIN48	-eGFR < 60ml/min	69/20	0.66	NA	NA
			U		-eGFR 60-<90 ml/min	116/25	0.44	NA	NA
			U		-eGFR 90-<120 ml/min	128/26	0.65	NA	NA
			U		-eGFR > 120 ml/min	66/11	0.37	NA	NA
		KIM-1	U	RRT		381/12	0.63	NA	NA
Metzger[63]	2010	KIM-1	U	AKI	AKIN crea	20/9	0.71	NA	NA
Liangos[39]	2007	KIM-1	U	RRT +M	↑Screa 0.5 mg/ dl	201/96	0.61	NA	NA
		NAG+KIM-1	U				0.71	NA	NA
		Apache + KIM-1					0.80	NA	NA
Westhuyzen[97]	2003	α GST	U	ARF	Screa * 1.5 and ↑ ≥ 0.15 mmol/l	26/4	0.89	60	95
		π GST	U				0.93	67	100
		γGT	U				0.95	67	100
		AP	U				0.86	67	90
		NAG	U				0.85	50	100
		LDH	U				0.69	100	96
		Cr Cl					0.80	50	91
Iglesias[98]	2003	IL-6	U	ARF	Screa > 3,5 mg/dl or RRT need If baseline Screa 1.8-3mg/dl: Screa*2 or RRT need	537/112	NA	NA	NA
		TNF-α	U						
		sTNFR-I	U						
		sTNFR-II	U						

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Herget-Rosenthal[84]	2004	α GST	U	RRT	*patients with non oliguric ATN*	73/26	0.64	NA	NA
		γGT	U				0.64	NA	NA
		NAG	U				0.81	55	88
		α_1MG	U				0.86	72	93
		RBP	U				0.80	NA	NA
		β_2MG	U				0.51	NA	NA
		LDH	U				0.59	NA	NA
		Liano score	U				0.83	63	85
Liangos[39]	2007	NAG	U	RRT +M	*Inclusion of patients with ARF defined as* BL ≤1.9mg/dl: ↑Screa 0.5 mg/ dl BL 2-4.9mg/dl: ↑Screa 1 mg/dl BL ≥5mg/dl: ↑Screa 1.5 mg/dl	201/96	0.71	NA	NA
		Screa enrollment					0.60	NA	NA
		Urine output					0.65	NA	NA
		Apache score					0.78	NA	NA
		Apache + NAG					0.79	NA	NA
Liu[99]	2007	Clinical model		AKI	Screa * 1.5 within 4 days	876/209	0.66	NA	NA
		Clinical model +biomarkers PAI-1 and sTNFR-I (not included in model, because not predictive: IL-6,IL-8,IL-10,TNF alfa,vWF,sTNRF-II, IADM-1)	P				0.70	NA	NA

		Clinical Model Clinical Model + biomarkers		AKI	Screa*1.5 day 1		0.72 0.77	NA NA	NA NA
Chawla Lakhmir[54]	2007	IL-6	P	AKI	Screa*1.25 or ↑0.3mg/dl during first week	547/127	NA	NA	NA
Hei[87]	2008	β2-MG NAG β2-MG Creatinine	U U S S	ARF	↑Screa to 132 µmol/l or ↑BUN to 18 mmol/l	60/10	NA NA NA NA	15 21 24 100	79 90 87 88
Walshe[100]	2009	αGST πGST	U	AKI	AKIN both	38/19	Biomarker not a good predictor of AKI	NA	NA
Portal[88]	2010	Creatinine eGFR APACHEII SOFA Creatinine eGFR APACHE II SOFA	S P U S 	AKI Severe AKI 	AKIN crea Severe AKI: ≥ stage 2 	80/30 80/19 	0.72 0.71 0.74 0.67 0.81 0.77 0.87 0.75	NA NA NA NA NA NA NA NA	NA NA NA NA NA NA NA NA
Metzger[63]	2010	MP ICU-T MP ICU-V	U U	AKI	AKIN both 2/16 AKIN crea	30/16 20/9	0.91 0.84	94 80	93 90

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Matsui[101]	2010	LFABP	U	AKI	AKIN crea	25/14	0.95	1	85
		NAG	U				0.63	70	53
		Albumine	U				0.70	77	67
		PCX	U				0.42	80	50
Endre[62]	2011	γGT	U	AKIN48	AKIN crea	381 (no AKI on entry)/82	0.57	NA	NA
		AP	U				0.56	NA	NA
		γGT	U	RIFLE 24hrs	RIFLE crea, sustained for	381/27	0.61	NA	NA
		AP	U		≥24h within 7 days		0.64	NA	NA
		γGT	U	AKIN48	-eGFR < 60ml/min	69/20	0.79	NA	NA
			U		-eGFR 60-<90 ml/min	116/25	0.48	NA	NA
					-eGFR 90-<120 ml/min	128/26	0.50	NA	NA
		AP	U		-eGFR > 120 ml/min	66/11	0.56	NA	NA
			U		-eGFR < 60ml/min	69/20	0.71	NA	NA
			U		-eGFR 60-<90 ml/min	116/25	0.48	NA	NA
			U		-eGFR 90-<120 ml/min	128/26	0.54	NA	NA
			U		-eGFR > 120 ml/min	66/11	0.55	NA	NA
		γGT		RRT		381/12	0.63	NA	NA
		AP					0.72	NA	NA

Author	PY	Biomarker	U/S/P	Outcome	AKI def / Outcome def	Patients / Events	AURoC	PPV (%)	NPV (%)
Blasco[102]	2011	γGT	U	AKI	CrCl 25% lower than theoretical	100/36	0.86	80	88
		Creatinine	P		CrCl based on age and gender		0.85	70	87
		γGT + Creatinine					NA	66	100

Abbreviations: Same as in tables 2-4. Not defined in tables 2-4: ATN: Acute tubular Necrosis, AKI sust: sustained AKI, M: Mortality, Rday-2/-1: 1 and 2 days before AKI diagnosed by serum creatinine, IDay -2/-1: 1 and 2 days before AKI diagnosed by serum creatinine, FDay -2/-1: 1 and 2 days before AKI diagnosed by serum creatinine, at adm: at admission, after adm: after admission, γGT: γ Glutamyl Transferase, AP: Alkaline Phosphatase, LDH: Lactate Dehydrogenase, RBP: Retinol Binding Protein, PAI-1: Plasminogen Activator Inhibitor-1, sTNFR-I and II: soluble Tumor Necrosis Factor receptor I and II, IADM-1: Intracellular Adhesion Molecule 1, TNFα: Tumor Necrosis Factor alpha, vWF; von Willebrand Factor, IL-10: Interleukin-10, MP ICU-T: Marker Pattern ICU Training set, MP ICU-V: Marker Pattern ICU Validation Set, pNGAL: plasma NGAL, PCX: podocalyxin, BL: baseline serum creatinine

Table 6: Contrast induced nephropathy

Author	PY	Biomarker	U/S/P	Outcome	AKI def/Outcome def	Patients/Events	AURoC	PPV (%)	NPV (%)
Nakamura[103]	2006	L-FABP	U	CIN	↑Screa >0.5mg/dl or *1.25 within 2-5 days	66/13	NA	NA	NA
Bachorzewska-Gajewska[104]	2007	NGAL	U	CIN	Screa*1.25 within 48h	100/11	NA	NA	NA
		NGAL	S				NA	NA	NA
Ling[64]	2008	NGAL	U	CIN	↑Screa 0.3 mg/dl: or * 1.25	40/13	0.73	20	97
Shaker[105]	2010	NGAL	S	CIN	Screa*1.25	30/2	NA	NA	NA
Bulent Gul[65]	2008	IL-18	U	CIN	↑Screa >0.5mg/dl or *1.25	51/15	NA	NA	NA
Ling[64]	2008	IL-18	U	CIN	↑Screa 0.3 mg/dl or * 1.25	40/13	0.75	20	96
Kato[106]	2008	Cystatin C	P	CIN	↑Screa 0,5 mg/dl or * 1.25 within 48h	87/18	0.93	63	98
		Creatinine	S				0.83	42	89
Briguori[107]	2010	Cystatin C	S	CIN	↑ Screa 0.3 mg/dl 48h PP or RRT	410/34	0.92	39	100

Ishibashi[108]	2010	Cystatin C	S	CIN	↑Screa 0,5 mg/dl or * 1.25	100/18	> 0.50	68	96
		Creatinine	S		within 48h		> 0.50	NA	NA
Bachorzewska-Gajewska[104]	2007	Cystatin C	S	CIN	Screa*1.25 within 48h	100/11	NA	NA	NA
Shaker[105]	2010	Cystatin C	S	CIN	Screa*1.25	30/2	NA	NA	NA

Abbreviations: Same as in tables 2-5. Not defined in tables 2-5: PP= Post Procedure

3.7 Reference List

1. Brivet FG, Kleinknecht DJ, Loirat P, Landais PJ. Acute renal failure in intensive care units--causes, outcome, and prognostic factors of hospital mortality; a prospective, multicenter study. French Study Group on Acute Renal Failure. *Crit Care Med* 1996; 24: 192-198

2. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005; 16: 3365-3370

3. Lameire N, Van BW, Vanholder R. Acute renal failure. *Lancet* 2005; 365: 417-430

4. Coca SG, Yalavarthy R, Concato J, Parikh CR. Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney Int* 2008; 73: 1008-1016

5. Lameire NH, Vanholder RC, Van Biesen WA. How to use biomarkers efficiently in acute kidney injury. *Kidney Int* 2011; 79: 1047-1050

6. Bennett M, Dent CL, Ma Q *et al*. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. *Clin J Am Soc Nephrol* 2008; 3: 665-673

7. Dennen P, Altmann C, Kaufman J *et al*. Urine interleukin-6 is an early biomarker of acute kidney injury in children undergoing cardiac surgery. *Crit Care* 2010; 14: R181

8. Dent CL, Ma Q, Dastrala S *et al*. Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. *Crit Care* 2007; 11: R127

9. Krawczeski CD, Vandevoorde RG, Kathman T *et al*. Serum cystatin C is an early predictive biomarker of acute kidney injury after pediatric cardiopulmonary bypass. *Clin J Am Soc Nephrol* 2010; 5: 1552-1557

10. Krawczeski CD, Woo JG, Wang Y, Bennett MR, Ma Q, Devarajan P. Neutrophil gelatinase-associated lipocalin concentrations predict development of acute kidney injury in neonates and children after cardiopulmonary bypass. *J Pediatr* 2011; 158: 1009-1015

11. Liu KD, Altmann C, Smits G *et al*. Serum interleukin-6 and interleukin-8 are early biomarkers of acute kidney injury and predict prolonged mechanical ventilation in children undergoing cardiac surgery: a case-control study. *Crit Care* 2009; 13: R104

12. Mishra J, Dent C, Tarabishi R *et al*. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet* 2005; 365: 1231-1238

13. Parikh CR, Devarajan P, Zappitelli M *et al*. Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. *J Am Soc Nephrol* 2011; 22: 1737-1747

14. Portilla D, Dent C, Sugaya T *et al*. Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery. *Kidney Int* 2008; 73: 465-472

15. Zappitelli M, Krawczeski CD, Devarajan P *et al*. Early postoperative serum cystatin C predicts severe acute kidney injury following pediatric cardiac surgery. *Kidney Int* 2011; 80: 655-662

16. Parikh CR, Mishra J, Thiessen-Philbrook H *et al*. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. *Kidney Int* 2006; 70: 199-203

17. Hirsch R, Dent C, Pfriem H *et al*. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. *Pediatr Nephrol* 2007; 22: 2089-2095

18. Du Y, Zappitelli M, Mian A *et al*. Urinary biomarkers to detect acute kidney injury in the pediatric emergency center. *Pediatr Nephrol* 2011; 26: 267-274

19. Haase-Fielitz A, Bellomo R, Devarajan P *et al*. The predictive performance of plasma neutrophil gelatinase-associated lipocalin (NGAL) increases with grade of acute kidney injury. *Nephrol Dial Transplant* 2009; 24: 3349-3354

20. Koyner JL, Bennett MR, Worcester EM *et al*. Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. *Kidney Int* 2008; 74: 1059-1069

21. Nickolas TL, O'Rourke MJ, Yang J *et al*. Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. *Ann Intern Med* 2008; 148: 810-819

22. Nickolas TL, Schmidt-Ott KM, Canetta P *et al*. Diagnostic and prognostic stratification in the emergency department using urinary biomarkers of nephron damage: a multicenter prospective cohort study. *J Am Coll Cardiol* 2012; 59: 246-255

23. Shapiro NI, Trzeciak S, Hollander JE *et al*. The diagnostic accuracy of plasma neutrophil gelatinase-associated lipocalin in the prediction of acute kidney injury in emergency department patients with suspected sepsis. *Ann Emerg Med* 2010; 56: 52-59

24. Soto K, Coelho S, Rodrigues B *et al*. Cystatin C as a marker of acute kidney injury in the emergency department. *Clin J Am Soc Nephrol* 2010; 5: 1745-1754

25. Bolignano D, Lacquaniti A, Coppolino G *et al*. Neutrophil gelatinase-associated lipocalin as an early biomarker of nephropathy in diabetic patients. *Kidney Blood Press Res* 2009; 32: 91-98

26. Brunner HI, Mueller M, Rutherford C *et al*. Urinary neutrophil gelatinase-associated lipocalin as a biomarker of nephritis in childhood-onset systemic lupus erythematosus. *Arthritis Rheum* 2006; 54: 2577-2584

27. Parravicini E, Nemerofsky SL, Michelson KA *et al*. Urinary neutrophil gelatinase-associated lipocalin is a promising biomarker for late onset culture-positive sepsis in very low birth weight infants. *Pediatr Res* 2010; 67: 636-640

28. Trachtman H, Christen E, Cnaan A *et al*. Urinary neutrophil gelatinase-associated lipocalcin in D+HUS: a novel marker of renal injury. *Pediatr Nephrol* 2006; 21: 989-994

29. Bolignano D, Lacquaniti A, Coppolino G, Campo S, Arena A, Buemi M. Neutrophil gelatinase-associated lipocalin reflects the severity of renal impairment in subjects affected by chronic kidney disease. *Kidney Blood Press Res* 2008; 31: 255-258

30. Mitsnefes MM, Kathman TS, Mishra J *et al*. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in children with chronic kidney disease. *Pediatr Nephrol* 2007; 22: 101-108

31. Haase M, Devarajan P, Haase-Fielitz A *et al*. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. *J Am Coll Cardiol* 2011; 57: 1752-1761

32. Constantin JM, Futier E, Perbet S *et al*. Plasma neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in adult critically ill patients: a prospective study. *J Crit Care* 2010; 25: 176

33. Cruz DN, de CM, Garzotto F *et al*. Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. *Intensive Care Med* 2010; 36: 444-451

34. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Dobrzycki S. Neutrophil-gelatinase-associated lipocalin and renal function after percutaneous coronary interventions. *Am J Nephrol* 2006; 26: 287-292

35. Bachorzewska-Gajewska H, Poniatowski B, Dobrzycki S. NGAL (neutrophil gelatinase-associated lipocalin) and L-FABP after percutaneous coronary interventions due to unstable angina in patients with normal serum creatinine. *Adv Med Sci* 2009; 54: 221-224

36. Nejat M, Pickering JW, Devarajan P *et al*. Some biomarkers of acute kidney injury are increased in pre-renal acute injury. *Kidney Int* 2012; 81: 1254-1262

37. Bucaloiu ID, Kirchner HL, Norfolk ER, Hartle JE, Perkins RM. Increased risk of death and de novo chronic kidney disease following reversible acute kidney injury. *Kidney Int* 2012; 81: 477-485

38. Coca S, Singanamala S, Parikh CR. Chronic kidney disease after acute kidney injury: a systematic review and meta-analysis. *Kidney International* 2012; 81: 442-448

39. Liangos O, Perianayagam MC, Vaidya VS *et al*. Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *J Am Soc Nephrol* 2007; 18: 904-912

40. Singer E, Elger A, Elitok S *et al*. Urinary neutrophil gelatinase-associated lipocalin distinguishes pre-renal from intrinsic renal failure and predicts outcomes. *Kidney Int* 2011; 80: 405-414

41. Washburn KK, Zappitelli M, Arikan AA *et al*. Urinary interleukin-18 is an acute kidney injury biomarker in critically ill children. *Nephrol Dial Transplant* 2008; 23: 566-572

42. Wheeler DS, Devarajan P, Ma Q *et al*. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. *Crit Care Med* 2008; 36: 1297-1303

43. Zappitelli M, Washburn KK, Arikan AA *et al*. Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. *Crit Care* 2007; 11: R84

44. Han WK, Wagener G, Zhu Y, Wang S, Lee HT. Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery. *Clin J Am Soc Nephrol* 2009; 4: 873-882

45. Koyner JL, Vaidya VS, Bennett MR *et al*. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. *Clin J Am Soc Nephrol* 2010; 5: 2154-2165

46. Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT. Urinary neutrophil gelatinase-associated lipocalin and acute kidney injury after cardiac surgery. *Am J Kidney Dis* 2008; 52: 425-433

47. Liangos O, Tighiouart H, Perianayagam MC *et al*. Comparative analysis of urinary biomarkers for early detection of acute kidney injury following cardiopulmonary bypass. *Biomarkers* 2009; 14: 423-431

48. Haase M, Bellomo R, Story D, Davenport P, Haase-Fielitz A. Urinary interleukin-18 does not predict acute kidney injury after adult cardiac surgery: a prospective observational cohort study. *Crit Care* 2008; 12: R96

49. Perry TE, Muehlschlegel JD, Liu KY *et al*. Plasma neutrophil gelatinase-associated lipocalin and acute postoperative kidney injury in adult cardiac surgical patients. *Anesth Analg* 2010; 110: 1541-1547

50. Haase-Fielitz A, Bellomo R, Devarajan P *et al*. Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery--a prospective cohort study. *Crit Care Med* 2009; 37: 553-560

51. McIlroy DR, Wagener G, Lee HT. Neutrophil gelatinase-associated lipocalin and acute kidney injury after cardiac surgery: the effect of baseline renal function on diagnostic performance. *Clin J Am Soc Nephrol* 2010; 5: 211-219

52. Devarajan P. Update on mechanisms of ischemic acute kidney injury. *J Am Soc Nephrol* 2006; 17: 1503-1520

53. Nakamura T, Sugaya T, Koide H. Urinary liver-type fatty acid-binding protein in septic shock: effect of polymyxin B-immobilized fiber hemoperfusion. *Shock* 2009; 31: 454-459

54. Chawla LS, Seneff MG, Nelson DR *et al*. Elevated plasma concentrations of IL-6 and elevated APACHE II score predict acute kidney injury in patients with severe sepsis. *Clin J Am Soc Nephrol* 2007; 2: 22-30

55. Bagshaw SM, Bennett M, Haase M *et al*. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. *Intensive Care Med* 2010; 36: 452-461

56. Siew ED, Ware LB, Gebretsadik T *et al*. Urine neutrophil gelatinase-associated lipocalin moderately predicts acute kidney injury in critically ill adults. *J Am Soc Nephrol* 2009; 20: 1823-1832

57. de Geus HR, Bakker J, Lesaffre EM, le Noble JL. Neutrophil gelatinase-associated lipocalin at ICU admission predicts for acute kidney injury in adult patients. *Am J Respir Crit Care Med* 2011; 183: 907-914

58. Perianayagam MC, Seabra VF, Tighiouart H, Liangos O, Jaber BL. Serum cystatin C for prediction of dialysis requirement or death in acute kidney injury: a comparative study. *Am J Kidney Dis* 2009; 54: 1025-1033

59. Nejat M, Pickering JW, Walker RJ *et al*. Urinary cystatin C is diagnostic of acute kidney injury and sepsis, and predicts mortality in the intensive care unit. *Crit Care* 2010; 14: R85

60. Siew ED, Ikizler TA, Gebretsadik T *et al*. Elevated urinary IL-18 levels at the time of ICU admission predict adverse clinical outcomes. *Clin J Am Soc Nephrol* 2010; 5: 1497-1505

61. Ferguson MA, Vaidya VS, Waikar SS *et al*. Urinary liver-type fatty acid-binding protein predicts adverse outcomes in acute kidney injury. *Kidney Int* 2010; 77: 708-714

62. Endre ZH, Pickering JW, Walker RJ *et al*. Improved performance of urinary biomarkers of acute kidney injury in the critically ill by stratification for injury duration and baseline renal function. *Kidney Int* 2011;

63. Metzger J, Kirsch T, Schiffer E *et al*. Urinary excretion of twenty peptides forms an early and accurate diagnostic pattern of acute kidney injury. *Kidney Int* 2010; 78: 1252-1262

64. Ling W, Zhaohui N, Ben H *et al*. Urinary IL-18 and NGAL as early predictive biomarkers in contrast-induced nephropathy after coronary angiography. *Nephron Clin Pract* 2008; 108: c176-c181

65. Bulent Gul CB, Gullulu M, Oral B *et al*. Urinary IL-18: a marker of contrast-induced nephropathy following percutaneous coronary intervention? *Clin Biochem* 2008; 41: 544-547

66. Hemdahl AL, Gabrielsen A, Zhu C *et al*. Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. *Arterioscler Thromb Vasc Biol* 2006; 26: 136-142

67. Parikh CR, Coca SG, Thiessen-Philbrook H *et al*. Postoperative biomarkers predict acute kidney injury and poor outcomes after adult cardiac surgery. *J Am Soc Nephrol* 2011; 22: 1748-1757

68. Xin C, Yulong X, Yu C, Changchun C, Feng Z, Xinwei M. Urine neutrophil gelatinase-associated lipocalin and interleukin-18 predict acute kidney injury after cardiac surgery. *Ren Fail* 2008; 30: 904-913

69. Wagener G, Jan M, Kim M *et al*. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. *Anesthesiology* 2006; 105: 485-491

70. Tuladhar SM, Puntmann VO, Soni M, Punjabi PP, Bogle RG. Rapid detection of acute kidney injury by plasma and urinary neutrophil gelatinase-associated lipocalin after cardiopulmonary bypass. *J Cardiovasc Pharmacol* 2009; 53: 261-266

71. Prabhu A, Sujatha DI, Ninan B, Vijayalakshmi MA. Neutrophil gelatinase associated lipocalin as a biomarker for acute kidney injury in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass. *Ann Vasc Surg* 2010; 24: 525-531

72. Haase M, Bellomo R, Devarajan P *et al*. Novel biomarkers early predict the severity of acute kidney injury after cardiac surgery in adults. *Ann Thorac Surg* 2009; 88: 124-130

73. Koyner JL, Garg AX, Coca SG *et al*. Biomarkers Predict Progression of Acute Kidney Injury after Cardiac Surgery. *J Am Soc Nephrol* 2012; 23: 905-914

74. Heise D, Rentsch K, Braeuer A, Friedrich M, Quintel M. Comparison of urinary neutrophil glucosaminidase-associated lipocalin, cystatin C, and alpha1-microglobulin for early detection of acute renal injury after cardiac surgery. *Eur J Cardiothorac Surg* 2011; 39: 38-43

75. Ristikankare A, Poyhia R, Kuitunen A *et al*. Serum cystatin C in elderly cardiac surgery patients. *Ann Thorac Surg* 2010; 89: 689-694

76. Wald R, Liangos O, Perianayagam MC *et al*. Plasma cystatin C and acute kidney injury after cardiopulmonary bypass. *Clin J Am Soc Nephrol* 2010; 5: 1373-1379

77. Liang XL, Liu SX, Chen YH *et al*. Combination of urinary kidney injury molecule-1 and interleukin-18 as early biomarker for the diagnosis and progressive assessment of acute kidney injury following cardiopulmonary bypass surgery: a prospective nested case-control study. *Biomarkers* 2010; 15: 332-339

78. Jorres A, Kordonouri O, Schiessler A *et al*. Urinary excretion of thromboxane and markers for renal injury in patients undergoing cardiopulmonary bypass. *Artif Organs* 1994; 18: 565-569

79. da Silva Magro MC, de FF, V. Does urinalysis predict acute renal failure after heart surgery? *Ren Fail* 2004; 26: 385-392

80. Eijkenboom JJ, van Eijk LT, Pickkers P, Peters WH, Wetzels JF, van der Hoeven HG. Small increases in the urinary excretion of glutathione S-transferase A1 and P1 after cardiac surgery are not associated with clinically relevant renal injury. *Intensive Care Med* 2005; 31: 664-667

81. Liangos O, Kolyada A, Tighiouart H, Perianayagam MC, Wald R, Jaber BL. Interleukin-8 and acute kidney injury following cardiopulmonary bypass: a prospective cohort study. *Nephron Clin Pract* 2009; 113: c148-c154

82. Gueret G, Lion F, Guriec N *et al*. Acute renal dysfunction after cardiac surgery with cardiopulmonary bypass is associated with plasmatic IL6 increase. *Cytokine* 2009; 45: 92-98

83. Ahlstrom A, Tallgren M, Peltonen S, Pettila V. Evolution and predictive power of serum cystatin C in acute renal failure. *Clin Nephrol* 2004; 62: 344-350

84. Herget-Rosenthal S, Poppen D, Husing J *et al*. Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. *Clin Chem* 2004; 50: 552-558

85. Herget-Rosenthal S, Marggraf G, Husing J *et al*. Early detection of acute renal failure by serum cystatin C. *Kidney Int* 2004; 66: 1115-1122

86. Mazul-Sunko B, Zarkovic N, Vrkic N *et al*. Proatrial natriuretic peptide (1-98), but not cystatin C, is predictive for occurrence of acute renal insufficiency in critically ill septic patients. *Nephron Clin Pract* 2004; 97: c103-c107

87. Hei ZQ, Li XY, Shen N, Pang HY, Zhou SL, Guan JQ. Prognostic values of serum cystatin C and beta2 microglobulin, urinary beta2 microglobulin and N-acetyl-beta-D-glucosaminidase in early acute renal failure after liver transplantation. *Chin Med J (Engl)* 2008; 121: 1251-1256

88. Portal AJ, McPhail MJ, Bruce M *et al*. Neutrophil gelatinase--associated lipocalin predicts acute kidney injury in patients undergoing liver transplantation. *Liver Transpl* 2010; 16: 1257-1266

89. Nejat M, Pickering JW, Walker RJ, Endre ZH. Rapid detection of acute kidney injury by plasma cystatin C in the intensive care unit. *Nephrol Dial Transplant* 2010; 25: 3283-3289

90. Royakkers AA, Korevaar JC, van Suijlen JD *et al*. Serum and urine cystatin C are poor biomarkers for acute kidney injury and renal replacement therapy. *Intensive Care Med* 2011; 37: 493-501

91. Niemann CU, Walia A, Waldman J *et al*. Acute kidney injury during liver transplantation as determined by neutrophil gelatinase-associated lipocalin. *Liver Transpl* 2009; 15: 1852-1860

92. Makris K, Markou N, Evodia E *et al*. Urinary neutrophil gelatinase-associated lipocalin (NGAL) as an early marker of acute kidney injury in critically ill multiple trauma patients. *Clin Chem Lab Med* 2009; 47: 79-82

93. Aghel A, Shrestha K, Mullens W, Borowski A, Tang WH. Serum neutrophil gelatinase-associated lipocalin (NGAL) in predicting worsening renal function in acute decompensated heart failure. *J Card Fail* 2010; 16: 49-54

94. Martensson J, Bell M, Oldner A, Xu S, Venge P, Martling CR. Neutrophil gelatinase-associated lipocalin in adult septic patients with and without acute kidney injury. *Intensive Care Med* 2010; 36: 1333-1340

95. Yang HN, Boo CS, Kim MG, Jo SK, Cho WY, Kim HK. Urine neutrophil gelatinase-associated lipocalin: an independent predictor of adverse outcomes in acute kidney injury. *Am J Nephrol* 2010; 31: 501-509

96. Parikh CR, Abraham E, Ancukiewicz M, Edelstein CL. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. *J Am Soc Nephrol* 2005; 16: 3046-3052

97. Westhuyzen J, Endre ZH, Reece G, Reith DM, Saltissi D, Morgan TJ. Measurement of tubular enzymuria facilitates early detection of acute renal impairment in the intensive care unit. *Nephrol Dial Transplant* 2003; 18: 543-551

98. Iglesias J, Marik PE, Levine JS. Elevated serum levels of the type I and type II receptors for tumor necrosis factor-alpha as predictive factors for ARF in patients with septic shock. *Am J Kidney Dis* 2003; 41: 62-75

99. Liu KD, Glidden DV, Eisner MD *et al*. Predictive and pathogenetic value of plasma biomarkers for acute kidney injury in patients with acute lung injury. *Crit Care Med* 2007; 35: 2755-2761

100. Walshe CM, Odejayi F, Ng S, Marsh B. Urinary glutathione S-transferase as an early marker for renal dysfunction in patients admitted to intensive care with sepsis. *Crit Care Resusc* 2009; 11: 204-209

101. Matsui K, Kamijo-Ikemori A, Hara M *et al*. Clinical significance of tubular and podocyte biomarkers in acute kidney injury. *Clin Exp Nephrol* 2010;

102. Blasco V, Wiramus S, Textoris J *et al*. Monitoring of plasma creatinine and urinary gamma-glutamyl transpeptidase improves detection of acute kidney injury by more than 20%. *Crit Care Med* 2011; 39: 52-56

103. Nakamura T, Sugaya T, Node K, Ueda Y, Koide H. Urinary excretion of liver-type fatty acid-binding protein in contrast medium-induced nephropathy. *Am J Kidney Dis* 2006; 47: 439-444

104. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E *et al*. Could neutrophil-gelatinase-associated lipocalin and cystatin C predict the development of contrast-induced nephropathy after percutaneous coronary interventions in patients with stable angina and normal serum creatinine values? *Kidney Blood Press Res* 2007; 30: 408-415

105. Shaker OG, El-Shehaby A, El-Khatib M. Early diagnostic markers for contrast nephropathy in patients undergoing coronary angiography. *Angiology* 2010; 61: 731-736

106. Kato K, Sato N, Yamamoto T, Iwasaki YK, Tanaka K, Mizuno K. Valuable markers for contrast-induced nephropathy in patients undergoing cardiac catheterization. *Circ J* 2008; 72: 1499-1505

107. Briguori C, Visconti G, Rivera NV *et al*. Cystatin C and contrast-induced acute kidney injury. *Circulation* 2010; 121: 2117-2122

108. Ishibashi Y, Yamauchi M, Musha H, Mikami T, Kawasaki K, Miyake F. Impact of contrast-induced nephropathy and cardiovascular events by serum cystatin C in renal insufficiency patients undergoing cardiac catheterization. *Angiology* 2010; 61: 724-730

CHAPTER 4:

INFLUENCE OF SEVERITY OF ILLNESS ON NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN PERFORMANCE AS A MARKER OF ACUTE KIDNEY INJURY IN SEPSIS.

Jill Vanmassenhove[1], Griet Glorieux[1], Norbert Lameire[1], Eric Hoste[2,3], Annemieke Dhondt[1], Raymond Vanholder[1], Wim Van Biesen[1]

[1]Renal Division, Ghent University Hospital

[2]Intensive Care Unit, Ghent University Hospital, [3]Senior Clinical Investigator – Research Foundation Flanders

Paper currently under submission

4.1 Abstract

Background: The role of neutrophil gelatinase-associated lipocalin (NGAL) as a diagnostic marker for Acute Kidney Injury (AKI) in sepsis is still debated. We hypothesized that in sepsis, the performance of serum(s) and urinary(u) NGAL can be negatively impacted by severity of illness, and that both uNGAL and sNGAL can be increased, even in the absence of AKI.

Materials and methods: Hundred-seven patients with sepsis were included. Urinary NGAL and sNGAL were measured at admission (T0) and 4 hours (T4) and 24 hours later (T24). Transient and intrinsic AKI were respectively defined as AKI according to RIFLE, that did or did not recover to "no AKI" in the following 5 days. Patients were classified according to tertiles of CRP increase (CRP≤20.10mg/dl, CRP 20.11-30.7mg/dl and CRP ≥30.71mg/dl). The relationship between sNGAL and uNGAL was assessed by linear regression.

Results: Fifty-seven patients developed transient and 22 intrinsic AKI. Prevalence of transient and intrinsic AKI was higher in patients with vs without septic shock (OR 3.3, p=0.007). uNGAL was associated to sNGAL, and this with parallel slopes but different intercepts for AKI ($Y= 0.87*X+314.3, R^2= 0.31$) and no AKI ($Y= 0.87*X+20.1, R^2=0.38$). At T4, median uNGAL and sNGAL levels were higher in septic patients with vs without shock but this independent of AKI (545ng/ml vs 196ng/ml for uNGAL and 474ng/ml vs 287ng/ml for sNGAL (both p=0.003). Urinary and sNGAL levels increased with tertiles of CRP increase. Neither uNGAL nor sNGAL had discriminating value for differentiating AKI from no-AKI within these categories.

Conclusion: Serum and uNGAL levels are influenced by severity of illness, independent of AKI. There is a strong correlation between sNGAL and uNGAL levels in sepsis. Increased levels of uNGAL do not automatically imply tubular damage, but can also be due to overspill from the systemic circulation.

4.2 Introduction

Septic Acute Kidney Injury (AKI) is associated with worse outcome compared to non-septic AKI and is regarded as a distinct clinical entity.[1] The unacceptably high mortality rates associated with septic AKI are partly explained by an incomplete understanding of the pathophysiology and a delay in diagnosis.[2-6] The adagio that renal vasoconstriction is the key pathophysiological pathway in septic AKI has recently been questioned while immunological mechanisms and oxidative stress inducing microcirculatory changes and tubular damage have gained interest.[5;7] Early diagnosis of septic or non-septic AKI remains cumbersome because it relies on imperfect parameters such as serum creatinine while introduction of new serum and urinary biomarkers could hypothetically allow earlier diagnosis and better prognostication.[8-10] At present, NGAL (neutrophil-gelatinase-associated-lipocalin) has been the most frequently investigated biomarker for early diagnosis of AKI.[11]

The shift in paradigm towards a more prominent role for immunological mechanisms and microvascular changes rather than renal hypoperfusion and renal vasoconstriction in the pathophysiology of septic AKI has created controversy about the existence of so called 'transient acute kidney injury'.[12] The latter is considered to be a physiological response to a decrease in glomerular capillary perfusion without structural tubular injury. This controversy has been amplified by the fact that some studies found increased urinary NGAL levels in patients classified as having transient AKI, suggesting presence of subtle tubular structural

injury.[13;14] In addition, studies where a rise in biomarker level (either in serum or urine) without a rise in serum creatinine or a decrease in urinary output was found, resulted in speculation on the existence of a new entity called *subclinical AKI.*[12;15] However, serum NGAL levels can be increased in many other conditions beside acute kidney injury, such as inflammation.[16] Moreover, serum NGAL is filtered at the glomerular level so that its presence in urine can reflect either glomerular overspill and/or local tubular production induced by structural injury.[17-19]

The present study aims to characterize the origin of the raised serum and urine levels of NGAL in septic patients. We hypothesized that, as in sepsis patients the prevalence of AKI is related to severity of sepsis, which in turn is associated with an increase in urinary and serum NGAL levels, a correlation between both urinary and serum NGAL, and severity of illness could exist, independent of the presence of AKI.

4.3 Material and methods

Study cohort:

One hundred and seven consecutive patients with sepsis, severe sepsis or septic shock, admitted to the Ghent University Hospital between 12/01/2010 and 05/09/2010, were prospectively enrolled. Sepsis, severe sepsis or septic shock were defined according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference guidelines.[20] Briefly, sepsis was defined when two or more of the following conditions were present as a result of infection: 1) temperature > 38° or < 36°, 2) heart rate > 90 beats/min, 3) respiratory rate > 20 breaths/min or PaCO2 < 32 mmHg (<4,3 kPa) or 4) white blood cell count > 12000 cells/mm^3 or < 4000 cells/mm^3, or > 10% immature (band) forms. Severe sepsis was defined as sepsis associated with organ dysfunction, hypoperfusion or hypotension. Sepsis with shock was defined as sepsis with hypotension despite adequate fluid resuscitation or vasopressor need. Since only 4 patients were not classified as having either severe sepsis or septic shock, we combined sepsis and severe sepsis in a new cumulative category "sepsis without shock". Exclusion criteria were 1) a history of liver and/or kidney transplantation, 2) ICU stay less than 24 hours, 3) patients treated with chronic hemodialysis and 4) age< 17 years.

Fluid management and decision making for need of RRT were done by intensivists, who were blinded to the study, and according to protocols applied in the study hospital. The study was approved by the ethical committee of the Ghent University Hospital. Written informed consent was obtained from the patient or their next of kin. Research was adhered to the tenets of the Declaration of Helsinki.

Study definitions:

We defined AKI based on the worst of either serum creatinine or urinary output criteria according to RIFLE. [21] The urinary output criterion was based on 6 hour blocks, as described by Macedo et al. [22] Baseline serum creatinine was based on the most recent value before admission or was estimated with the MDRD equation if the latter was not available. [21]

Transient acute kidney injury was defined as presence of AKI according to RIFLE, occurring in the first three days after admission and returning to no AKI within 5 days. Intrinsic AKI was defined as presence of AKI according to RIFLE in the first three days of admission that did not

improve to no AKI in the following days. Patients who left ICU before 5 days after admission were followed-up at the department where they were transferred to.

Sample collection:

Urine and blood samples were collected at the moment of admission (T0), four hours later (T4) and 24 hours later (T24). Blood samples were centrifuged at 1500g for 10 minutes within 20 minutes after collection, and serum was aliquoted and stored at -80°C for later batch analysis. Urine was collected in a sterile way and centrifuged at 500g for 10 minutes, urine samples were aliquoted and stored at -80°C for later batch analysis.

Serum and Urinary Neutrophil-Gelatinase-Associated-Lipocalin (NGAL) were measured using an ELISA kit (Bioporto^{R} Diagnostics Denmark).

Data collection:

After informed consent, demographics and medical history were obtained. Laboratory and cilinical data were gathered. APACHE II scores were calculated over the first 24 hours of admission.

The highest CRP value in the first 5 days after admission was retained and patients were classified according to tertiles of CRP increase: CRP≤20.10mg/dl, CRP 20.11-30.70mg/dl and CRP ≥30.71mg/dl.

Statistical Analysis:

Results are reported as medians and interquartile ranges (IQR) for continuous variables, unless otherwise specified. Discrete variables are reported as numbers and/or percentages. All statistical analyses were performed using SPSS® 19. All consecutive patients fulfilling the inclusion criteria, were included, irrespective of their course or duration of stay at ICU.

Demographic characteristics of the study cohort were compared using using Mann-Whitney U test (two groups) or Kruskal Wallis (>two groups) in case of continuous variables that are not normally distributed. In case of continuous variables with a normal distribution, Student's t test (two groups) or one way ANOVA (>two groups) were used to compare means.

Patients were classified according to sepsis status (either sepsis without shock or sepsis with shock) and AKI status (no AKI vs transient AKI vs intrinsic AKI). The Kruskall Wallis test was used to compare median uNGAL and sNGAL levels between groups at the three different time points (T0,T4 and T24). For each sepsis category separately (sepsis without or with sohck), uNGAL and sNGAL levels were compared between no-AKI, transient AKI and intrinsic AKI at the different time point, using the Kruskall Wallis test. Dichotomous variables were compared between groups using Chi Square analysis.

Regression analysis was used to assess association between serum and urinary NGAL.

4.4 Results

Demographics and clinical background of the 107 included patients are presented in tables 1 and 2 as partially published elsewhere.[23] (Table 1 and Table 2). Four patients had sepsis

(3.7%), 38 had severe sepsis (35.5%) and 65 septic shock (60.7%). Twenty-eight (26.2%) patients were classified as having no-AKI versus 57(53.3%) and 22(20.6%) as having transient and intrinsic AKI, respectively. Median APACHE II score was 21 in patients without shock and 23 in those with shock (p=0.22) and increased from no-AKI over transient AKI to intrinsic AKI (p=0.08).

More sepsis patients with shock vs without shock had transient or intrinsic AKI (35/65 vs 22/42 and 19/65 vs 3/42, respectively, p=0.007). There was also an increasing positive fluid balance, need for ventilation, length of ICU stay and mortality from no AKI over transient AKI to intrinsic AKI and in patients with versus those without shock. Fourteen patients needed RRT. (Table 1 and Table 2) Median urinary and serum NGAL levels were higher in sepsis patients with vs those without shock. (Figure 1 A, Figure 1B, Figure 1 C and Table 3)

Table 1: Clinical and demographic characteristics of the study cohort comparing patients with no AKI vs transient AKI vs intrinsic AKI.

	no-AKI (n=28)	**transient AKI (n=57)**	**intrinsic AKI (n=22)**	**p value**
Gender male(%)	52	54	59	0.93
Age(years,mean/sd)	55.4(17.3)	62.6(13.2)	63.1(14.9)	0.08
CKD on admission (eGFR according to MDRD <60ml/min/1,73m2) (%)	11	9	9	1
APACHE II score on the first day of admission	21(10)	22(8)	24.5(9)	0.08
Fluid balance first 24 hours (mean/sd)	2.2(1.8)	3.1(1.9)	5.4(2.7)	<0.001
Use of diuretics on the first day of admission (%)	7	14	23	0.29
RRT need during ICU stay (%)	0	2	59	<0.001
Vasopressor use (%)	32	61	86	<0.001
Total dose of Noradrenaline first 24h in mg/kg (mean/sd)	0.08(0.16)	0.13(0.17)	0.29(0.32)	0.001
Maximum dose of Noradrenaline during first 24h in µg/kg/min (mean/sd)	0.12(0.23)	0.25(0.31)	0.54(0.55)	0.002
Need for ventilation during ICU stay (%)	39	51	86	<0.001
LOS in the ICU (days)	5(6)	5(10)	38(32)	0.014
ICU mortality (%)	21	16	54	0.002
Mortality at 90 days(%)	**29**	**25**	**59**	**0.012**

AKI: Acute Kidney Injury, CKD: Chronic Kidney Disease, eGFR according to MDRD equation: estimated glomerular filtration rate according to modification of diet in renal disease equation, APACHE II: Acute Physiology and Health Care Evaluation II, RRT: Renal Replacement Therapy, ICU: Intensive Care Unit, LOS: Length of Stay.

Table 2: Clinical and demographic characteristics of the study cohort comparing sepsis patients without vs with shock.

	sepsis without shock (n=42)	**sepsis with shock (n=65)**	**p value**
Gender male(%)	50	60	0.31
Age(years,mean/sd)	57.(15.6)	62.9(14.3)	0.07
CKD on admission (eGFR according to MDRD <60ml/min/1,73m2) (%)	17	5	0.04
APACHE II score on the first day of admission	21(9)	23(9)	0.22
AKI (transient or intrinsic) (%)	25(60)	54(83)	0.007
Fluid balance first 24 hours (mean/sd)	2.06(2.21)	3.8(2.58)	<0.001
Use of diuretics on the first day of admission (%)	10	17	0.28
RRT need during ICU stay (%)	0	22	0.001
Total dose of Noradrenaline first 24h in μg/kg/min (mean/sd)	N/A	0.17(0.16)	N/A
Maximum dose of Noradrenaline during first 24h in μg/kg/min (mean/sd)	N/A	0.44(0.41)	N/A
Need for ventilation during ICU stay (%)	39	74	<0.001
LOS in the ICU (days)	4(6)	7(16)	0.03
ICU mortality (%)	14	32	0.04
Mortality at 90 days(%)	19	42	0.02

AKI: Acute Kidney Injury, CKD: Chronic Kidney Disease, eGFR according to MDRD equation: estimated glomerular filtration rate according to modification of diet in renal disease equation, APACHE II: Acute Physiology and Health Care Evaluation II, RRT: Renal Replacement Therapy, N/A: Not Applicable, ICU: Intensive Care Unit, LOS: Length of Stay.

Figure 1: Urinary and serum NGAL in sepsis without vs with shock

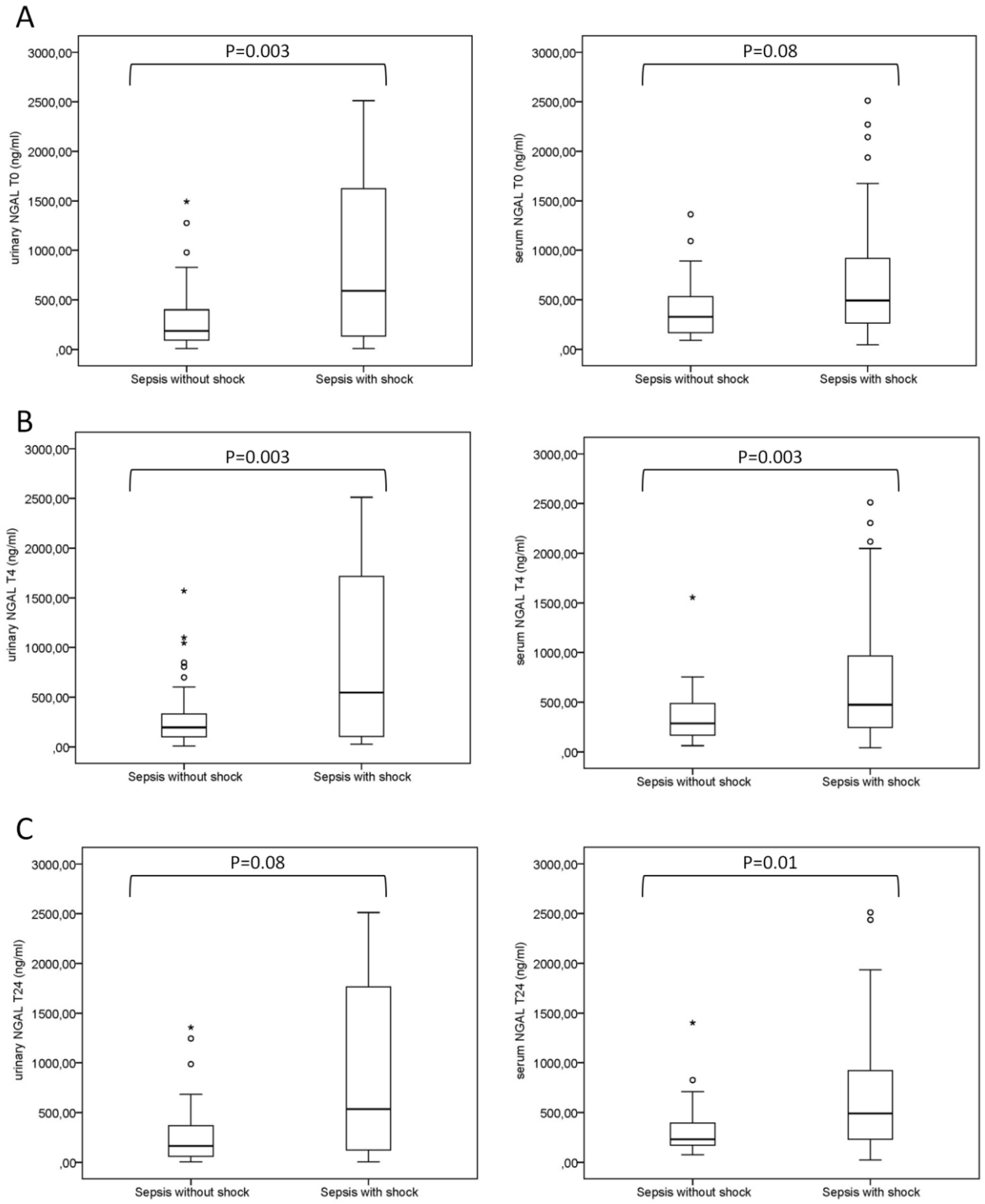


*Figure legends: A: Urinary NGAL(ng/ml) at time point T0 is higher in sepsis with vs without shock (p=0.003). There is a trend for higher serum NGAL levels (ng/ml) in sepsis with vs without shock (p=0.083). B: Serum and urinary NGAL (ng/ml) at time point T4 are higher in sepsis with shock vs without shock (both p=0.003). C: Serum NGAL(ng/ml) at time point T24 is higher in sepsis with vs without shock (p=0.011). There is a trend for higher urinary NGAL levels (ng/ml) in sepsis with vs without shock (p=0.082). (°=outliers; *=extreme outliers (> three times the height of the boxes))*

Table 3: Urinary NGAL and serum NGAL (ng/ml) in no-AKI vs transient and intrinsic AKI.

	Sepsis without shock				**Sepsis with shock**				
	no AKI (n=17)	transient AKI (n=22)	Intrinsic AKI (n=3)	p value	no AKI (n=11)	transient AKI (n=35)	intrinsic AKI (n=19)	p value	overall p value
uNGAL T0	125(262)	262(350)	1276	0.27*	178(457)	649(1164)	1775(2108)	0.08*	0.001#
uNGAL T4	116(256)	234(310)	1044	0.27*	269(511)	523(1384)	1802(2046)	0.03*	0.009#
uNGAL T24	122(218)	239(383)	1245	0.27*	137(612)	405(1523)	2372(2308)	0.11*	0.084#
sNGAL T0	220(269)	288(477)	235	0.44*	290(221)	493(579)	962(894)	0.003*	0.03#
sNGAL T4	218(193)	336(338)	284	0.17*	267(192)	469(604)	975(940)	0.011*	0.002#
sNGAL T24	203(189)	295(364)	184	0.44*	283(188)	475(435)	1052(670)	0.001*	0.001#

* p values refer to the difference between no AKI, transient AKI and intrinsic AKI, separately for patients with vs without shock.

p values refer to the difference between no AKI, transient AKI and intrinsic AKI either with or without shock at each time point.

All no-AKI patients had serum NGAL levels above the generally accepted cut-off of 150 ng/ml at all time points. All sepsis patients with shock had urinary NGAL levels above 150 ng/ml at admission and four hours later, even if they did not have AKI. Urinary and serum NGAL levels were higher in intrinsic vs transient vs no AKI patients, but there was substantial overlap limiting discriminative value. (Table 3) When classified according to sepsis without vs with shock, discriminative value of NGAL for AKI further decreased. (Figure 2 A, Figure 2 B and Figure 2 C)

Figure 2: Urinary NGAL in no-AKI, transient AKI and intrinsic AKI, stratified according to sepsis severity.

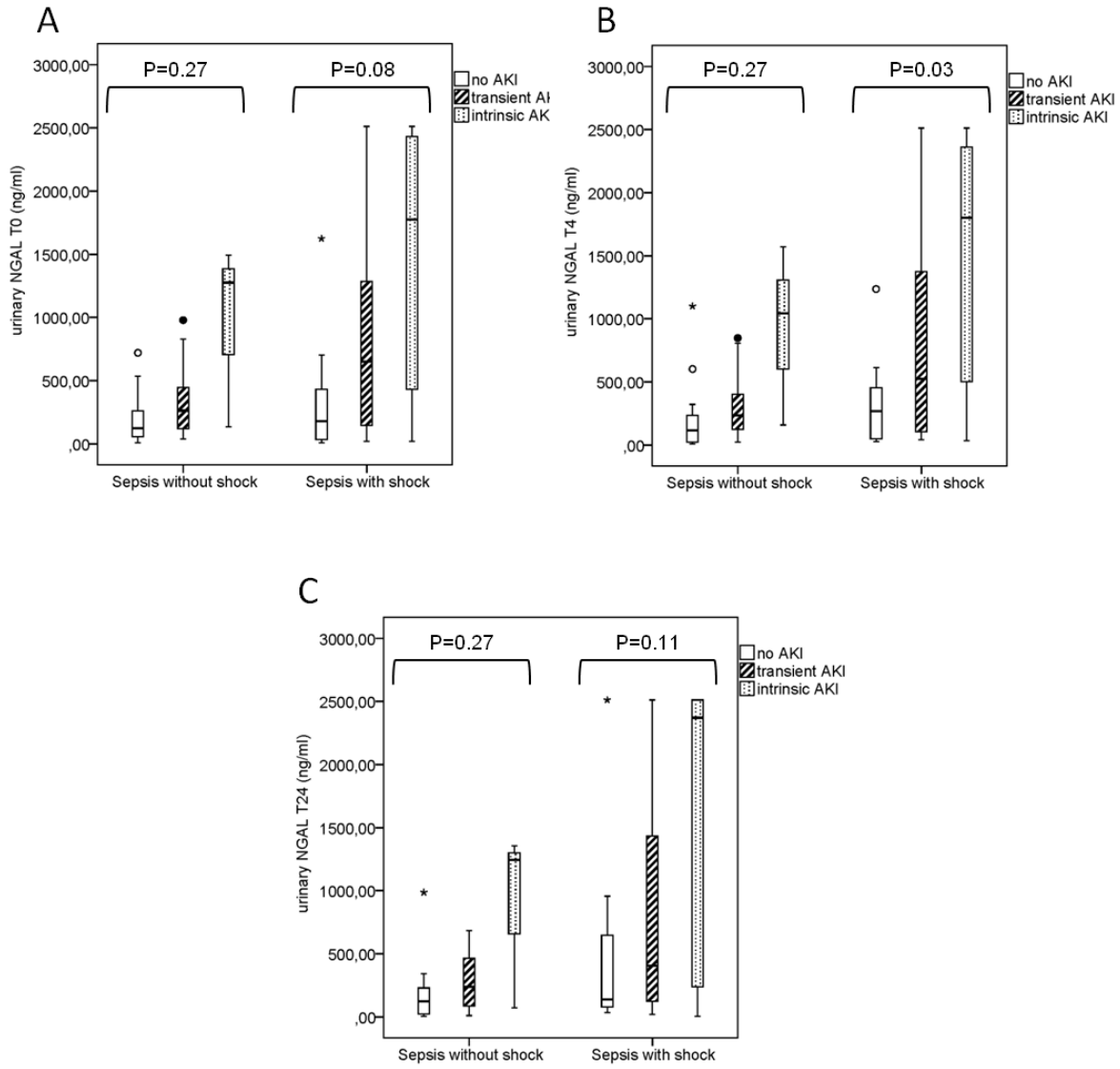


Figure legend: (A) At time point T0, urinary NGAL(ng/ml) is not significantly different between no-AKI, transient AKI and intrinsic AKI in sepsis without shock (p=0.27) and sepsis with shock (p=0.08). (B) At time point T4, urinary NGAL(ng/ml) is not significantly different between no-AKI, transient AKI and intrinsic AKI in sepsis without shock (p=0.27).(C) At time point T24, urinary NGAL(ng/ml) is not significantly different between no-AKI, transient AKI and intrinsic AKI in sepsis without shock (p=0.27) and sepsis with shock (p=0.11).

*(°=outliers; *=extreme outliers (> three times the height of the boxes))*

Urinary and serum NGAL levels increased with tertiles of CRP (175ng/ml vs 229ng/ml vs 563ng/ml, for uNGAL and 245ng/ml vs 296ng/ml vs 512ng/ml for sNGAL (p=0.006 and p=0.04, respectively). (figure 3 A and Figure 3 B) Neither uNGAL or sNGAL had a discriminative value for differentiating AKI (transient or intrinsic) from no AKI. (Figure 3 C and Figure 3 D)

Figure 3: Influence of inflammation on serum and urinary NGAL

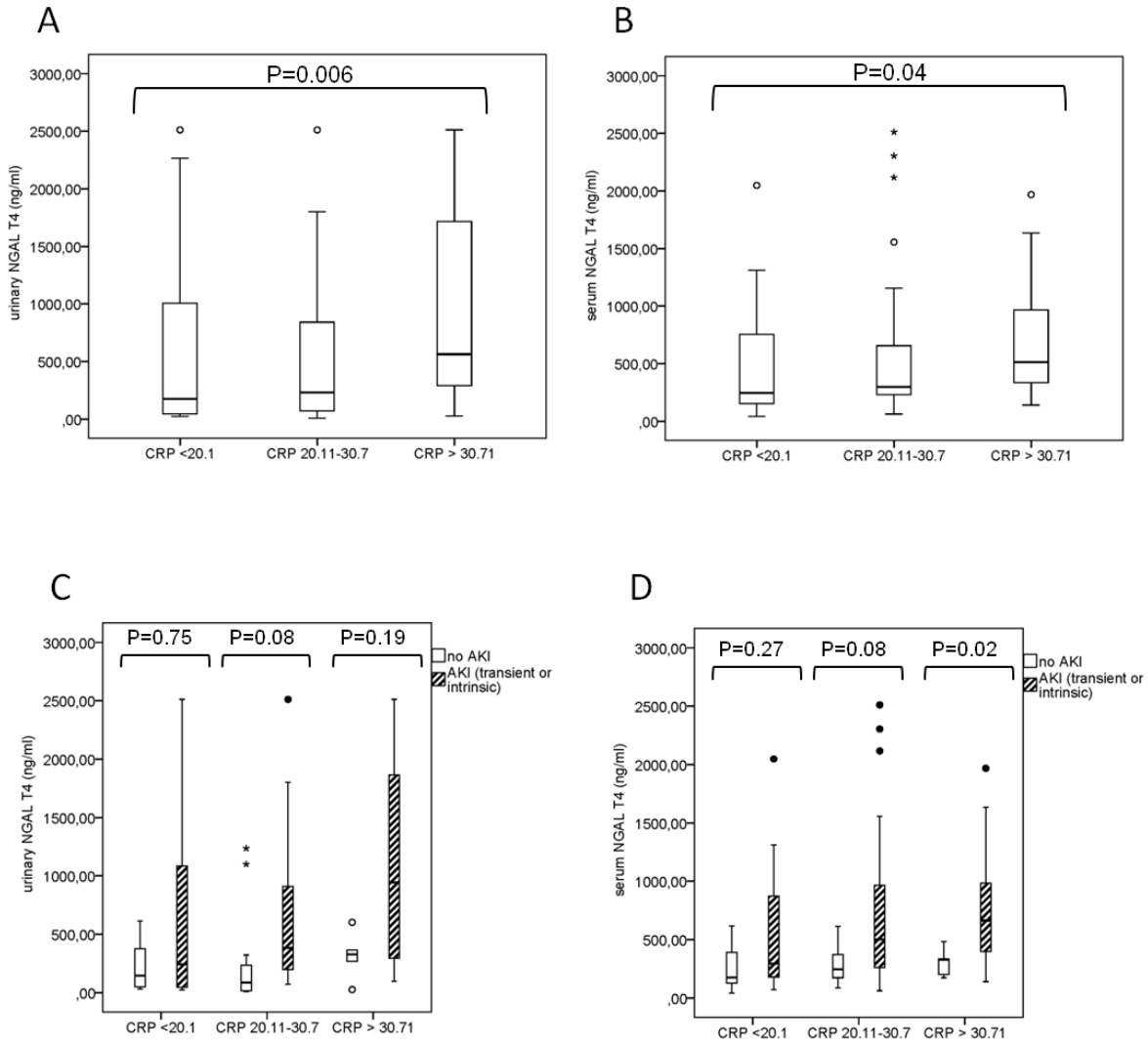
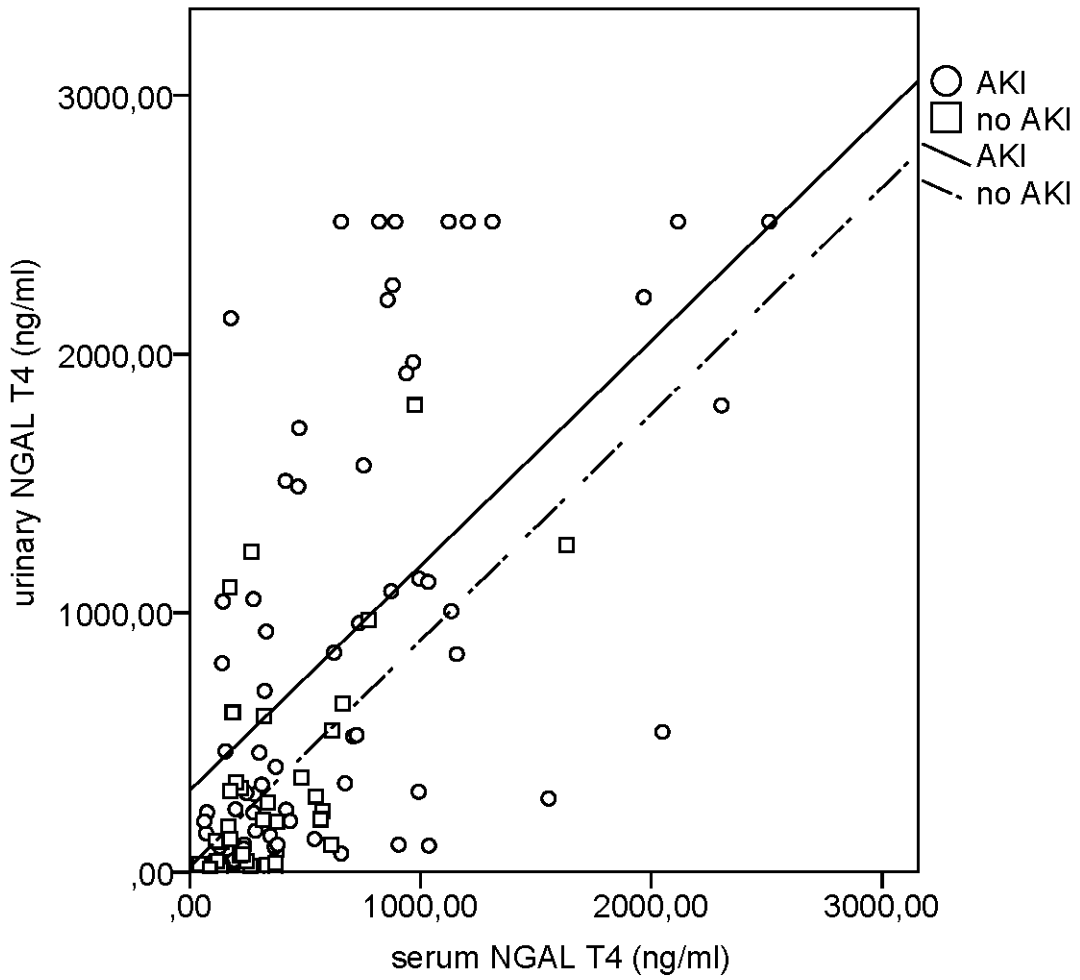


*Figure legend: (A): Urinary NGAL(ng/ml) increases together with increasing levels of CRP. (B): Serum NGAL(ng/ml) increases together with increasing levels of CRP. (C): Urinary NGAL(ng/ml) is not significantly different between AKI (transient or intrinsic) and no AKI, when stratified according to tertiles of CRP increase. (D) Serum NGAL(ng/ml) is not significantly different between AKI (transient or intrinsic) and no AKI in the two lower tertiles of CRP increase. (°=outliers; *=extreme outliers (> three times the height of the boxes))*

We found a strong correlation between sNGAL and uNGAL, both in patients without and with AKI (R^2=0.38 for no AKI and R^2= 0.31 for AKI), but with different relationships in no AKI (Y= 0.87*X+20.1) vs AKI (Y= 0.87*X+314.3), respectively (p< 0.001). The slopes of the regression lines followed a parallel course. (Figure 4)

Figure 4: Linear regression between serum NGAL and urinary NGAL in no AKI and AKI

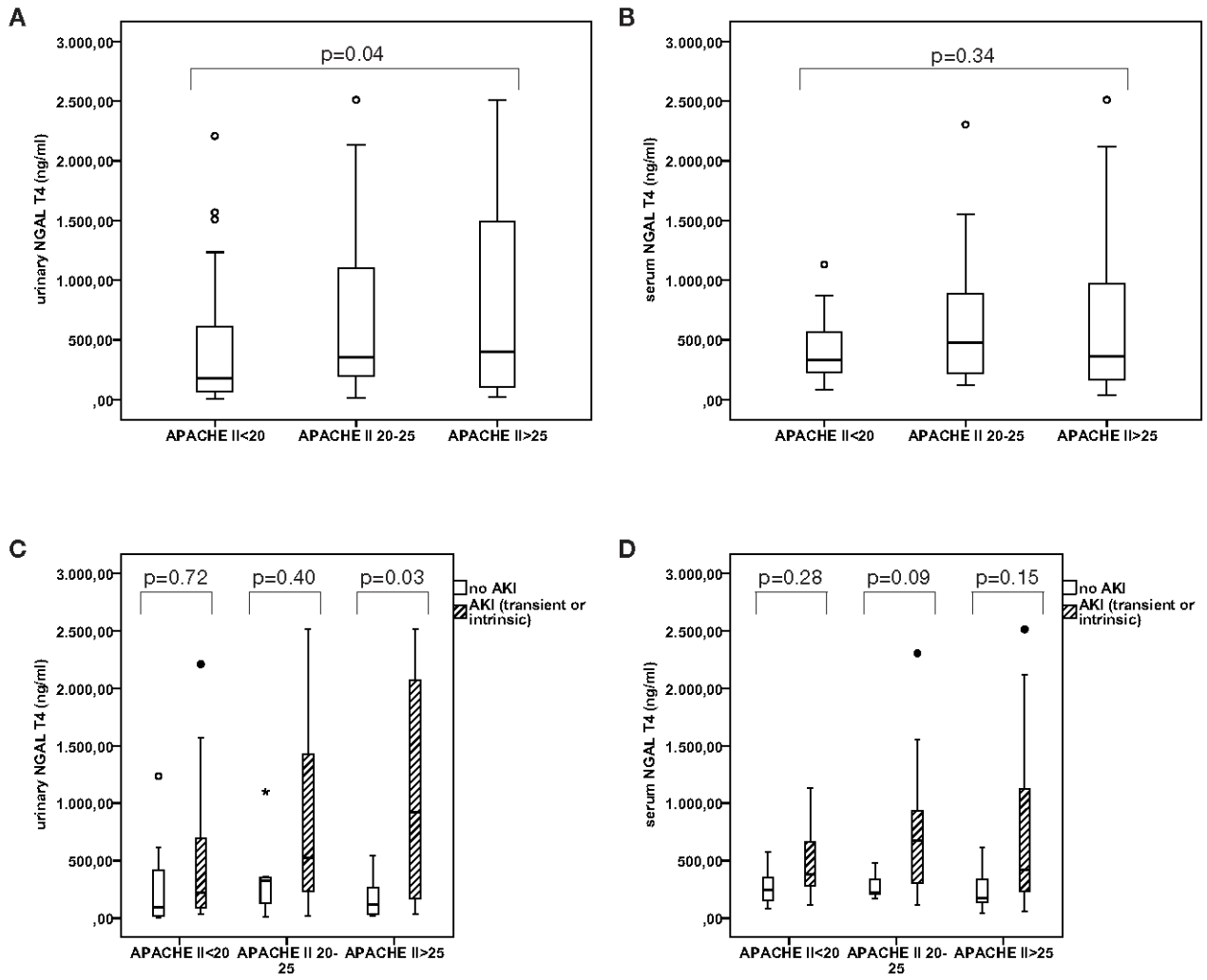


*Legend figure 4: For no AKI: y=0.87*x + 20.1 and for AKI: y=0.87*x+314.3. R^2 for AKI=0.31 and R2 for no-AKI=0.38 (p<0.001).*

Analyses of the correlation between sNGAL and uNGAL levels at the time points 4 and 24 hours demonstrated comparable findings. (data not shown)

There was also a correlation between the APACHE II score and uNGAL (p=0.002, p<0.001 and p=0.003 at T0, T4 and T24) and between the APACHE II score and sNGAL (p=0.007, p=0.003 and p=0.07 at T0,T4 and T24. Median urinary NGAL levels increased with increasing tertiles of APACHE II (179ng/ml vs 355ng/ml vs 405ng/ml for APACHE II<20, 20-25 and >25 respectively). There was an increasing trend in serum NGAL levels over the first two tertiles (Figure 5).

Figure 5: Influence of severity of illness on serum and urinary NGAL



Legend Figure 5: (A): Urinary NGAL(ng/ml) increases together with tertiles of APACHE II score increase. (B): There is a trend for increasing serum NGAL(ng/ml) together with APACHE II score increase, in the two lower tertiles of APACHE II score increase. (C): Urinary NGAL(ng/ml) is not significantly different between AKI (transient or intrinsic) and no AKI, in the two lower tertiles of APACHE II score increase.

4.5 Discussion

In this cohort of septic ICU patients, we confirmed that the risk for AKI increased with severity of sepsis. Serum and urinary levels of NGAL increased with severity of illness and inflammation, as assessed by APACHE II and CRP, and this irrespective of presence of AKI. In addition, there was a strong correlation between urinary and serum levels of NGAL, again irrespective of presence of AKI. Although there was a significant difference in uNGAL levels between no-AKI, transient AKI and intrinsic AKI, this difference did not remain when patients were stratified according to severity of sepsis (sepsis with or without shock) or to tertiles of CRP increase or APACHE II score, except at time point T4 in patients with shock.

As such, it remains unclear whether in sepsis patients, increased urinary NGAL is a marker of structural tubular injury, the result of overspill from the systemic circulation or just a marker of severity of illness.

NGAL is a 25 kD molecule which is filtered into the primary urine and almost completely reabsorbed by the tubular epithelium via the megalin receptor under normal circumstances [24]. One of the concerns of using NGAL as a biomarker for AKI is that even in the absence of AKI, NGAL levels can increase during inflammation.[16;25-29] We demonstrated that in patients with sepsis, serum NGAL levels increase in parallel with the severity of sepsis, severity of illness and severity of inflammation, irrespective of the presence of AKI. Because the prevalence of AKI is associated with severity of illness, a clear judgement on whether NGAL reflects severity of illness and/or inflammation rather than presence of AKI, is difficult.

High levels of serum NGAL can overwhelm the reabsorptive capacity of the proximal tubule so that urinary NGAL levels might increase, even in the absence of structural tubular injury. We found a strong correlation between serum and urinary NGAL levels, both in patients without and with AKI. The value of urinary NGAL for differentiating between AKI and no AKI was low due to overlap between the two groups, and this irrespective of severity of illness, sepsis or inflammation.

Thesefindings underline that the concept of "subclinical AKI" should be used with caution, however,they do not contradict the existence of this conceptin certain patients. Indeed, urinary NGAL was well correlated with serum NGAL ($R^2 = 0.37$), but the regression line went through the origin for no AKI patients, whereas it did not in AKI patients. This suggests that in AKI, there is some degree of either local tubular production, or reduced reabsorption, both of which may reflect tubular injury.

Although the question whether a patient has true "subclinical tubular injury" or just overspill from the circulation due to severity of illness and its associated risk for AKI might seem purely semantic, it has some consequences for clinical practice. The current evolution towards an increasing use of NGAL for diagnostic purposes because of its proposed discriminating role in differentiating AKI from no AKI should, based on the current data, be considered with care at least in patients with sepsis. In addition, if patients classified as having "subclinical AKI", based on NGAL positivity only, would be included in an interventional trial, interventions that only focus on preventing or healing tubular injury will appear to be less useful because in a substantial part of patients, the increased urinary NGAL will not be the result of tubular damage but rather of overspill from the circulation as a consequence of high circulating levels induced by inflammation.

A limitation of this observational study is that it describes a relatively small cohort of septic patients. However, to our knowledge, this is the first study providing information on prospectively collected serum and urinary NGAL levels at different time points during the first 24 hours after admission in septic patients.

4.6 Conclusion

In patients with sepsis, levels of urinary and serum NGAL and the prevalence and severity of AKI are strongly associated with severity of illness and inflammation as expressed by APACHE II and CRP. There is a strong correlation in sepsis patients between serum and urinary levels

of NGAL, and this irrespective of presence of AKI. Therefore, presence of NGAL in the urine does not automatically imply tubular injury in sepsis patients.

4.7 Reference List

1. Bagshaw SM, Uchino S, Bellomo R *et al*. Septic acute kidney injury in critically ill patients: clinical characteristics and outcomes. *Clinical Journal of The American Society of Nephrology: CJASN* 2007; 2: 431-439

2. Matejovic M, Radermacher P, Joannidis M. Acute kidney injury in sepsis: is renal blood flow more than just an innocent bystander? *Intensive Care Medicine* 2007; 33: 1498-1500

3. Molitoris BA. Renal blood flow in sepsis: a complex issue. *Critical Care (London, England)* 2005; 9: 327-328

4. Wan L, Bellomo R, Di GD, Ronco C. The pathogenesis of septic acute renal failure. *Curr Opin Crit Care* 2003; 9: 496-502

5. Wang Z, Holthoff JH, Seely KA *et al*. Development of oxidative stress in the peritubular capillary microenvironment mediates sepsis-induced renal microcirculatory failure and acute kidney injury. *American Journal of Pathology* 2012; 180: 505-516

6. Zarjou A, Agarwal A. Sepsis and acute kidney injury. [Review]. *Journal of the American Society of Nephrology* 2011; 22: 999-1006

7. Ishikawa K, May CN, Gobe G, Langenberg C, Bellomo R. Pathophysiology of septic acute kidney injury: a different view of tubular injury. *Contrib Nephrol* 2010; 165: 18-27

8. Cruz DN, Ricci Z, Ronco C. Clinical review: RIFLE and AKIN--time for reappraisal. *Crit Care* 2009; 13: 211

9. Doi K, Yuen PS, Eisner C *et al*. Reduced production of creatinine limits its use as marker of kidney injury in sepsis. *J Am Soc Nephrol* 2009; 20: 1217-1221

10. Endre ZH, Westhuyzen J. Early detection of acute kidney injury: emerging new biomarkers. *Nephrology (Carlton)* 2008; 13: 91-98

11. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 2009; 54: 1012-1024

12. Haase M, Kellum JA, Ronco C. Subclinical AKI--an emerging syndrome with important consequences. *Nat Rev Nephrol* 2012; 8: 735-739

13. Doi K, Katagiri D, Negishi K *et al*. Mild elevation of urinary biomarkers in prerenal acute kidney injury. *Kidney Int* 2012; 82: 1114-1120

14. Nejat M, Pickering JW, Devarajan P *et al*. Some biomarkers of acute kidney injury are increased in pre-renal acute injury. *Kidney Int* 2012; 81: 1254-1262

15. Ronco C, Kellum JA, Haase M. Subclinical AKI is still AKI. *Crit Care* 2012; 16: 313

16. Giasson J, Li GH, Chen Y. Neutrophil gelatinase-associated lipocalin (NGAL) as a new biomarker for non--acute kidney injury (AKI) diseases. *Inflamm Allergy Drug Targets* 2011; 10: 272-282

17. Axelsson L, Bergenfeldt M, Ohlsson K. Studies of the release and turnover of a human neutrophil lipocalin. *Scand J Clin Lab Invest* 1995; 55: 577-588

18. Mori K, Lee HT, Rapoport D *et al*. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. *J Clin Invest* 2005; 115: 610-621

19. Schmidt-Ott KM, Mori K, Li JY *et al*. Dual action of neutrophil gelatinase-associated lipocalin. *J Am Soc Nephrol* 2007; 18: 407-413

20. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20: 864-874

21. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; 8: R204-R212

22. Macedo E, Malhotra R, Claure-Del GR, Fedullo P, Mehta RL. Defining urine output criterion for acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2011; 26: 509-515

23. Vanmassenhove J, Glorieux G, Hoste E, Dhondt A, Vanholder R, Van BW. Urinary output and fractional excretion of sodium and urea as indicators of transient versus intrinsic acute kidney injury during early sepsis. *Crit Care* 2013; 17: R234

24. Hvidberg V, Jacobsen C, Strong RK, Cowland JB, Moestrup SK, Borregaard N. The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin with high affinity and mediates its cellular uptake. *FEBS Lett* 2005; 579: 773-777

25. Bagshaw SM, Bennett M, Haase M *et al*. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. *Intensive Care Med* 2010; 36: 452-461

26. Martensson J, Bell M, Oldner A, Xu S, Venge P, Martling CR. Neutrophil gelatinase-associated lipocalin in adult septic patients with and without acute kidney injury. *Intensive Care Med* 2010; 36: 1333-1340

27. Wheeler DS, Devarajan P, Ma Q *et al*. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. *Crit Care Med* 2008; 36: 1297-1303

28. Hur M, Kim H, Lee S *et al*. Diagnostic and prognostic utilities of multimarkers approach using procalcitonin, B-type natriuretic peptide, and neutrophil gelatinase-associated lipocalin in critically ill patients with suspected sepsis. *BMC Infect Dis* 2014; 14: 224

29. Kim H, Hur M, Cruz DN, Moon HW, Yun YM. Plasma neutrophil gelatinase-associated lipocalin as a biomarker for acute kidney injury in critically ill patients with suspected sepsis. *Clin Biochem* 2013; 46: 1414-1418

CHAPTER 5:

URINARY OUTPUT AND FRACTIONAL EXCRETION OF SODIUM AND UREA AS INDICATORS OF TRANSIENT VERSUS INTRINSIC AKI DURING EARLY SEPSIS.

Vanmassenhove J.[1], Glorieux G.[1], Hoste E.[2,3], Dhondt A.[1], Vanholder R.[1], Van Biesen W.[1]

[1]Renal Division, Ghent University Hospital

[2]Intensive Care Unit, Ghent University Hospital, [3]Senior Clinical Investigator – Research Foundation Flanders

Crit Care 2013, 17(5):R234.

5.1 Abstract

Introduction: The pathophysiology of acute kidney injury (AKI) in sepsis is ill defined. We investigated parameters associated with low glomerular filtration, and their predictive value to discriminate transient from intrinsic septic AKI.

Methods: In 107 sepsis patients, AKI was defined by the RIFLE urinary output or serum creatinine criterion, or both. Transient AKI (TAKI) vs intrinsic AKI was defined as RIFLE R, I or F on the first day evolving to no AKI or not, respectively, over the following five days. Fractional excretion of sodium (FENa), urea (FEUrea) and NGAL (FENGAL) at admission (d0t0), 4 (d0t4) and 24 hours (d1) were determined.

Results: Including vs not including the urinary output criterion of RIFLE increased AKI from 43 to 64,5%. Median uNGAL levels and FENGAL were lower in no AKI versus transient AKI when AKI was defined based on creatinine (p=0.002 and p=0.04 respectively) but not when based on urinary output (p=0.9 and p=0.49 respectively). FENa<1% and FEUrea <35% was present in 77.3 and 63.2 of patients. Urinary NGAL was higher (p<0.001) in those with high vs low fractional sodium excretion, but this only in patients with transient or intrinsic AKI (p<0.001 in subgroups), and not in patients without AKI. The negative predictive value for either intrinsic AKI or not restoring diuresis in patients with FENa>0.36% and FEUrea>31.5% was 92% and 94.5% respectively.

Conclusions: A low FENa and FEUrea is highly prevalent in the first hours of sepsis. In sepsis, oliguria is an earlier sign of impending AKI than increase in serum creatinine. A combination of a high FENa and a low FEUrea is associated with intrinsic AKI, whereas a combined high FENa and FEUrea are strongly predictive of transient AKI.

5.2 Introduction

There is controversy about the role of low glomerular filtration pressure in the pathogenesis of septic Acute Kidney Injury (AKI)[1-3]. Tubular damage due to inflammatory cascades is also forwarded as a potential underlying pathophysiological mechanism [4-7]. Recently, it was demonstrated that transient azotemia can be more than a physiologic response to low glomerular filtration pressure, as it can also be associated with transient low grade tubular injury, as defined by presence of Neutrophil Gelatinase-Associated Lipocalin (NGAL)[8] or Liver Fatty Acid Binding Protein (LFABP)[9]. Whereas these findings enhance our understanding of the pathophysiology of AKI, they are not always very helpful in clinical practice, where the most important dilemma is to differentiate those cases where restoration of glomerular filtration pressure can potentially still reverse AKI from those where it will only lead to fluid overload. This is important, as there is increasing evidence that links blind fluid loading to higher mortality.[10;11]

Although most definitions of AKI are based on both an increase in serum creatinine and a decrease in urinary output, the latter criterion has been neglected in many studies[12-15]. Recent work has demonstrated that urinary output is important, as it is associated with morbidity and mortality[16]. A low urinary output can be used as an early warning parameter of developing AKI, maybe even before tubular damage arises.

We hypothesized that the combined interpretation of a reduced urinary output, and a low fractional excretion of sodium and urea, and especially the evolution of these parameters, is an early indicator of incipient AKI in sepsis patients, at a moment that it is still reversible if glomerular filtration pressure is restored. We also hypothesized that a high fractional sodium excretion can be an indicator of active sodium secretion by damaged tubular cells and reduced proximal tubular reabsorption, whereby extent of tubular damage was assessed by (fractional excretion of) urinary neutrophil gelatinase associated lipocalin (NGAL).

5.3 Materials and methods

Ethics statement: The study was approved by the ethical committee of the Ghent University Hospital. Written informed consent was obtained from the patients or their next of kin.

107 consecutive patients with sepsis admitted to the intensive care unit (ICU) of a tertiary university hospital were included between 12/01/2010 and 5/09/2010. Sepsis, severe sepsis and septic shock were defined according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference guidelines[17]. Exclusion criteria were 1) a history of liver and/or kidney transplantation, 2) ICU stay less than 24 hours, 3) patients treated with chronic hemodialysis and 4) age< 17 years.

Acute Kidney Injury (AKI) was defined according to RIFLE classification (Risk, Injury, Failure, Loss of kidney function, and End stage renal disease)[13]. Baseline creatinine was based on the most recent value before admission or was calculated if the latter was not available[13]. The urinary output criterion was based on 6 hour blocks, as described by Macedo et al[16]. As we wanted to assess the impact of the creatinine and the urine output criterion separately, we defined AKI based on both criteria (AKIboth) together, versus on the urinary output criterion only (AKIuo), or the serum creatinine criterion alone (AKIc). Transient AKI (TAKI) was defined as RIFLE R, I or F on day 1 that improved to "no AKI" in the following five days, whereas "intrinsic AKI" was defined as patients with RIFLE R, I or F who did not evolve to no AKI in the following five days.

Urine and blood samples were collected at the moment of admission (time point d0t0), and also four hours later (time point d0t4) and 24 hours later (time point d1), to assess the evolution of the parameters. Blood samples were centrifuged within 20 minutes after collection at 1500g for 10 minutes, and serum was aliquoted and stored at -80°C for later batch analysis. Urine was collected in a sterile way and centrifuged at 500g for 10 minutes; urine samples were aliquoted and stored at -80°C for later batch analysis.

Serum and Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) were measured using an ELISA kit (Bioporto^{R} Diagnostics Denmark).

Fractional excretion of sodium, urea and NGAL was calculated according to the formula $(U_x x S_{crea})/(U_{crea} x S_x) x100$ with x=either sodium, urea or NGAL. Patients were classified according to FEUrea and FENa quartiles. We also defined FENA and FEUrea dichotomously as "high" (two upper quartiles) and "low" (two lower quartiles).

Statistical analysis

Results are reported as medians and interquartile ranges (IQR) for continuous variables, unless otherwise specified. Discrete variables are reported as numbers and/or percentages. All statistical analyses were performed using SPSS® 19. All consecutive patients were included, irrespective of their course or duration of stay at ICU.

Continuous variables were compared between groups using Mann-Whitney U test (two groups) or Kruskal Wallis (>2 groups), as appropriate, using the non-parametric section of SPSS®19. When the overall p-value was <0.05, post-hoc analysis between different pairs was performed using Mann-Whitney U tests, taking into account correction for multiple testing.

Dichotomous variables in groups were compared using Chi Square analysis. Positive and negative predictive values were calculated for high and low fractional excretion of sodium and urea, whereby "high" and "low" where based on the median value. We performed Receiver Operating Characteristics (RoC) curves to assess the performance of fractional excretion of sodium and urea to predict transient or intrinsic AKI.

Performance of the combined interpretation of fractional excretion of sodium and urea was evaluated by calculating positive and negative predictive power of the different combinations for restoration of diuresis and for development of intrinsic AKI.

5.4 Results

107 consecutive patients were included. Demographic and clinical data are shown separately for AKI vs no AKI patients as defined by RIFLE on the first day of admission in table 1.

Table 1: Clinical and demographic characteristics of the study cohort comparing patients without vs with AKI as defined by RIFLE on the first day of admission.

	No AKI (n=38)	**AKI (n=69)**	**p value**
Gender male(%)	57.9	55.1	0.78
Age(years,mean/sd)	57.5(16.0)	62.7(14.2)	0.09
CKD on admission (MDRD <60ml/min/1,73m2) (%)	10.5	8.7	0.76
APACHE II score on the first day of admission	21(10)	23(9)	0.19
Positive fluid balance first 24 hours (mean/sd)	2.5(2.1)	4.0(2.3)	0.002
Use of diuretics on the first day of admission (%)	18.4	17.4	0.89
RRT need during ICU stay (%)	5.3	17.4	0.08

Vasopressor use (%)	42.1	68.1	0.009
	No AKI (n=38)	**AKI (n=69)**	**p value**
Total dose of Noradrenaline first 24h in µg/kg/min	0.15(0.25)	0.13(0.20)	0.83
Maximum dose of Noradrenaline during first 24h in µg/kg/min	0.32(0.44)	0.34(0.51)	0.83
Need for ventilation during ICU stay (%)	47.4	59.4	0.23
LOS (days)	5(9)	6(12)	0.82
ICU mortality (%)	21.1	27.5	0.46
Mortality at 90 days(%)	26.3	36.2	0.30

Demographic and clinical data for no AKI vs transient AKI vs intrinsic AKI are shown in table 2.

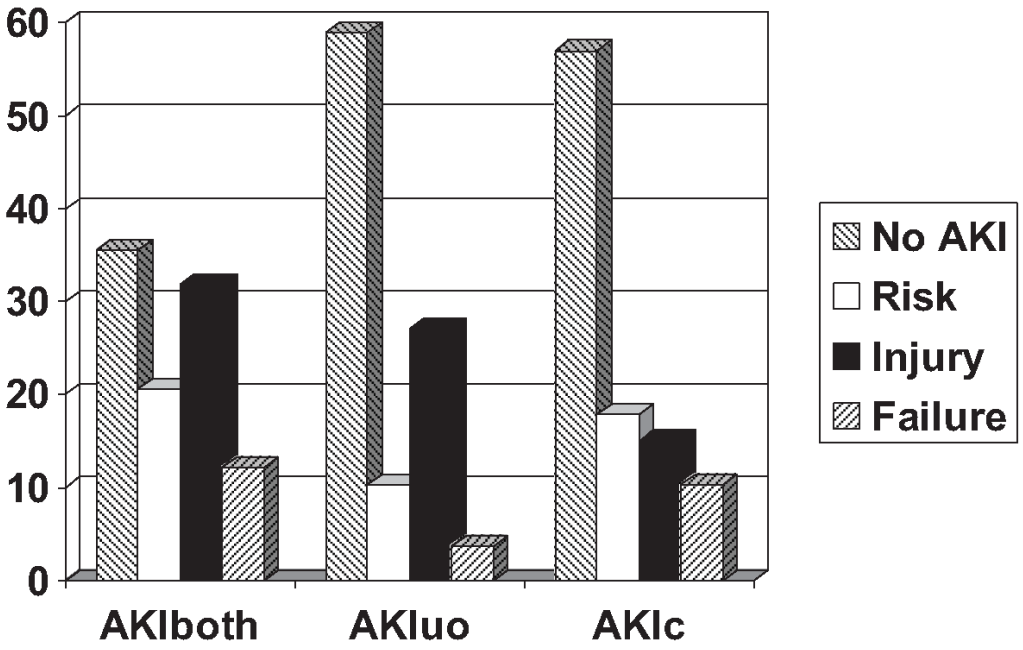
Table 2: Clinical and demographic characteristics of the study cohort comparing patients with no AKI vs transient AKI vs intrinsic AKI.

	No AKI (n=28)	**Transient AKI (n=57)**	**Intrinsic AKI(n=22)**	**p value**
Gender male(%)	51.7	54.4	59.1	0.93
Age(years,mean/sd)	55.4(17.3)	62.6(13.3)	63.1(14.9)	0.08
CKD on admission (MDRD <60ml/min/1,73m2) (%)	10.7	8.8	9.1	0.96
APACHE II score on the first day of admission	21(10)	22(8)	24.5(9)	0.08
Positive fluid balance first 24 hours (mean/sd)	2.2(1.8)	3.3(1.9)	5.4(2.7)	<0.001
Use of diuretics on the first day of admission (%)	7.1	19.3	27.3	0.16
RRT need during ICU stay (%)	0	1.8	59.1	<0.001
Vasopressor use (%)	32.1	61.4	86.4	<0.001
Total dose of Noradrenaline first 24h in µg/kg/min	0.13(0.22)	0.12(0.19)	0.16(0.28)	0.65
Maximum dose of Noradrenaline during first 24h in µg/kg/min	0.28(0.34)	0.32(0.47)	0.49(0.68)	0.66
Need for ventilation during ICU stay (%)	39.3	50.9	86.4	0.003

LOS (days)	5(6)	5(10)	38(32)	0.014
ICU mortality (%)	21.4	15.8	54.2	0.002
Mortality at 90 days(%)	28.6	24.6	59.1	0.012

Based on both RIFLE criteria combined (AKIboth), 35.5% of patients had no AKI, and 20.6%, 31.8% and 12.1% had RIFLE R, I and F. When AKI was defined based on urinary output alone (AKIuo), 58.9% patients were classified as having no-AKI, and 10.3%, 27.1% and 3.7% as RIFLE-R, I or F respectively. When defining AKI based on the creatinine criterion alone (AKIc), 57% had no AKI, and 17.8%, 15% and 10.3% were classified as RIFLE R, I and F (figure1). So, omitting the urinary output criterion leads to underdiagnosis of AKI.

Figure 1: Distribution of RIFLE class based on a single or both criteria.



Legend Figure 1: Based on the urinary and the creatinine criteria together (AKIboth), 35.5% of patients had no AKI, versus 20.6%, 31.8% and 12.1% with RIFLE R, I and F. Based on the urinary output criterion only (AKIuo), 58.9% patients were classified as having no-AKI, versus 10.3%, 27.1% and 3.7% as RIFLE-R, I or F respectively. Based on the creatinine criterion only (AKIc), 57% had no AKI, versus 17.8%, 15% and 10.3% classified as RIFLE R, I and F (p 0.03).

Upper cut-off values for the FENa quartiles based on the values of d0t0 were 0.15%, 0.36%, 0.95%. At time points d0t0, d0t4 and d1, 77.3, 74.5 and 71.1% of patients had a fractional excretion of sodium (FENa) < 1%, the currently used cut-off value.

Upper cut-off values for the FEUrea quartiles were 21%, 31.5%, and 42.0%. At time points d0t0, d0t4 and d1, 63.2%, 50.9% and 41.3% of patients had a fractional excretion of urea (FEUrea) <35%.

There was a stepwise increase in prevalence of AKI based on urinary output with quartiles of decreasing FENa and FEUrea at d0t0 (p 0.05 and p 0.01) and d0t4 ($p < 0.001$ for both), but not at d1 for FENa ($p=0.18$). There was no such association for AKI based on creatinine (table 3).

Table 3: Prevalence of AKIuo and AKIc (% of all patients classified in that category) in different FENa and FEUrea quartiles

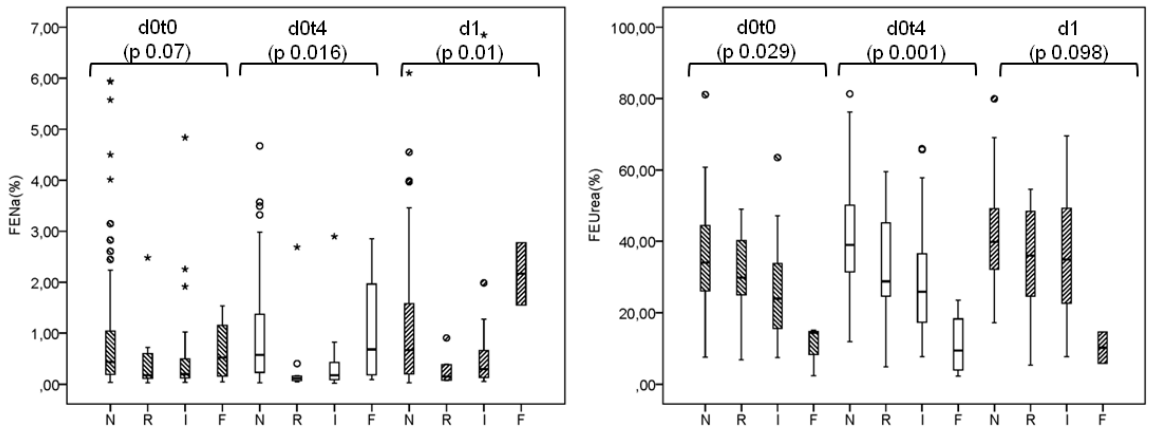
FENa quartiles	**AKIuo d0t0 p=0.05**	**AKIuo d0t4 p<0.001**	**AKIuo d1 p=0.18**
<0.15	57.7	72.4	53.3
0.15-0.36	48.1	34.6	46.7
0.36-0.95	33.3	39.1	33.3
>0.95	23.1	14.3	28.1
FEUrea quartiles	AKIuo d0t0 p=0.01	AKIuo d0t4 p<0.001	AKIuo d1 p=0.01
<21	59.3	77.8	83.3
21.35	48.1	55.5	38.1
31.5-42	37.5	22.2	30
>42	17.9	23.5	34.1

FENa quartiles	**AKIc d0t0 p=0.76**	**AKIc d0t4 p=0.66**	**AKIc d1 p=0.09**
<0.15	34.6	37.9	33.3
0.15-0.36	40.7	46.2	40
0.36-0.95	48.1	34.8	29.6
>0.95	46.2	50	59.4
FEUrea quartiles	AKIc d0t0 p=0.06	AKIc d0t4 p=0.17	AKIc d1 p=0.72
<21	55.6	61.1	33.3
21.35	51.9	44.4	33.3
31.5-42	20.8	44.4	46.7
>42	39.3	29.4	43.9

p values represent Chi square over the quartiles.

The values for FENa and FEUrea in the different AKI stages based on urinary output are illustrated in figure 2A and figure 2B, respectively. FENa was <1% in the majority of patients at all time points, except in patients classified as 'F' at timepoint d1, where the median was 2.16%. There was a U-shaped pattern of FENa over the different AKI stages based on urinary output (p=0.07, 0.016 and 0.01 at d0t0, d0t4 and d1 respectively)(figure 2A). There was a decreasing trend in FEUrea at d0t0,d0t4 and d1 across different AKI stages based on urinary output (figure 2B). Patients with stage 'F'AKIuo had a persistent FEUrea <20% at the three time points (figure 2 B).

Figure 2: FENa(%)(A) and FEUrea(%)(B) at the different time points in AKIuo



Legend Figure 2: A: U-shaped form of FENa over the different AKIuo classes(N= no AKI, R=Risk, I=Injury and F=Failure). (p=0.07, 0.016 and 0.01 at d0t0, d0t4 and d1 respectively). B: Decreasing trend in FEUrea(%) across AKIuo classes at the different time points with persistently low FEUrea(%) in RIFLE 'F' class at the three time points (p 0.029, p0.001 and p 0.098).

To analyse the discriminative power of fractional excretion of sodium and urea and of urinary NGAL to discriminate transient and intrinsic AKI, we performed Receiver Operating Characteristic curves, yielding areas under the curves of 0.59, 0.36 and 0.67 respectively, pointing out that none of these parameters had sufficient power to discriminate intrinsic from transient AKI.

We wanted to assess the predictive value of a combined interpretation of fractional excretion of sodium and urea for the evolution of diuresis and AKI. Therefore, a categorical representation of percentages of patients with no AKI, transient AKI and intrinsic AKI in different subgroups of high and low fractional excretion of sodium and urea at 4 hours is cross tabulated in table 4 (A: based on urinary output: p<0.001, B: based on creatinine: p=0.01, C: based on both urinary output and creatinine: p<0.001).

Table 4: cross tabulation of FENa<0.36%/FEurea<31.5% vs FENa>0.36%/FEurea<31.5% vs FENa<0.36%/FEurea>31.5% vs FENa>0.36%/FEurea>31.5% in AKIuo (Table 4A), AKIc (Table 4B) and AKIboth (Table 4C).

A

AKIuo	**FENa<0.36%**	**FENa>0.36%**	**FENa<0.36%**	**FENa>0.36%**	**Total**
% within FENA/FEUrea category	**FEUrea<31.5%**	**FEUrea<31.5%**	**FEUrea>31.5%**	**FEUrea>31.5%**	
No AKI	18.8	7.7	43.5	71.8	42.1
Transient AKI	62.5	38.5	52.2	23.1	43.0

Intrinsic AKI	18.8	53.8	4.3	5.1	15.0

B

AKIc	**FENa<0.36%**	**FENa>0.36%**	**FENa<0.36%**	**FENa>0.36%**	**Total**
% within FENA/FEUrea category	**FEUrea<31.5%**	**FEUrea<31.5%**	**FEUrea>31.5%**	**FEUrea>31.5%**	
No AKI	46.9	30.8	65.2	56.4	52.3
Transient AKI	34.4	15.4	21.7	35.9	29.9
Intrinsic AKI	18.8	53.8	13.0	7.7	17.8

C

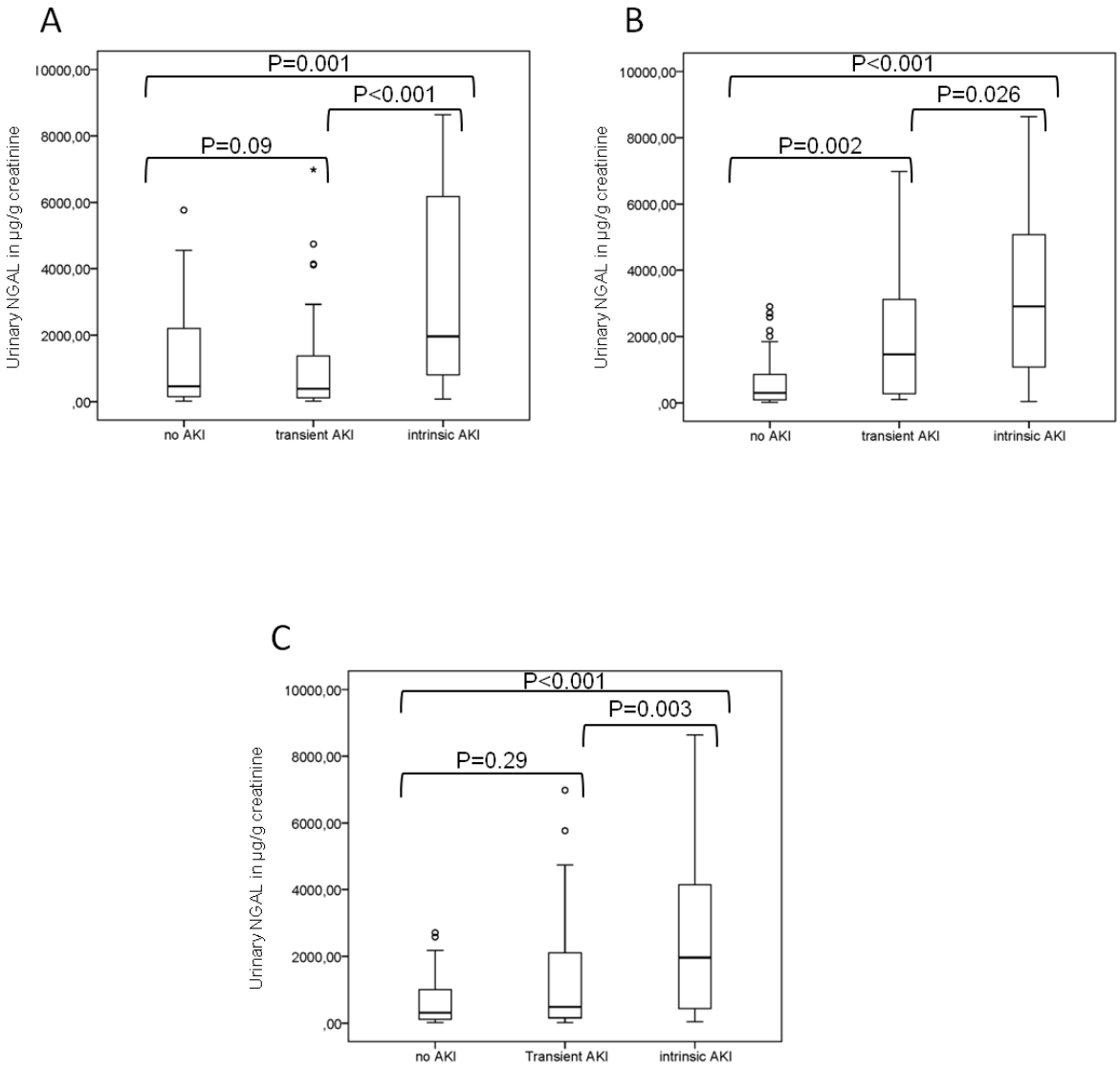
AKIboth	**FENa<0.36%**	**FENa>0.36%**	**FENa<0.36%**	**FENa>0.36%**	**Total**
% within FENA/FEUrea category	**FEUrea<31.5%**	**FEUrea<31.5%**	**FEUrea>31.5%**	**FEUrea>31.5%**	
No AKI	9.4	0.0	30.4	46.2	26.2
Transient AKI	68.8	38.5	52.2	46.2	53.3
Intrinsic AKI	21.9	61.5	17.4	7.7	20.6

AKIuo, AKIc, AKIboth: Acute kidney injury based on urinary output(uo), serum creatinine(c) or both criteria(both).A: p<0.001, B: p=0.01 and C: p<0.001

The negative predictive value for intrinsic AKI for patients with both high fractional excretion of sodium and urea was 92.0%; the negative predictive value that urinary output would not restore in patients with this combination was 94.9%. In contrast, the positive predictive value of a high fractional sodium excretion in combination with a low fractional urea excretion for persisting oliguria was 54%. Thus, a high fractional sodium and urea excretion are nearly always associated with transient AKI and restoration of diuresis, whereas in patients with a high fractional sodium excretion in combination with a low fractional urea excretion, there is substantial risk for persisting oliguria. The same evaluation for the same parameters at admission showed the same trend (data not shown).

Urinary NGAL values at 4 hours in patients with no AKI, transient AKI and intrinsic AKI are represented in figure 3, and this separately for AKI defined on urinary output (figure 3A), on creatinine (figure 3B) or both criteria (figure 3C) (p value overall<0.001).

Figure 3: uNGAL in no-AKI, transient AKI and intrinsic AKI based on AKIuo(A), AKIc(B) and AKIboth(C).



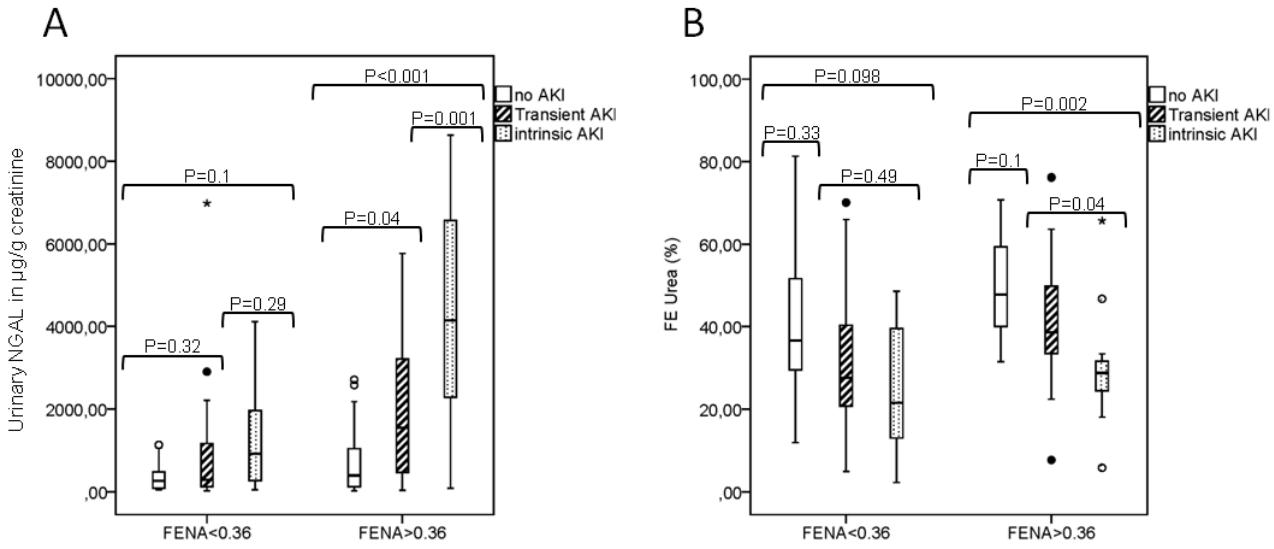
Legend Figure 3: Overall p value was <0.001 for the difference in uNGAL levels between groups, in figure 3A,3B and 3C. There was no significant difference (p=0.9) in uNGAL levels between transient AKI and no-AKI if diagnosis was based on the urinary output criterion (figure 3A). If diagnosis was based on the creatinine criterion (figure 3B), there was a significant difference between no-AKI and transient AKI (p 0.002).

In post hoc analysis, there was a difference in median NGAL levels between no AKI and transient AKI when AKI was defined based on creatinine (p=0.002) whereas there was no difference when it was based on urinary output (p=0.9). There was a similar gradual increase in the fractional excretion of NGAL in no-AKI versus transient AKI (p=0.04) versus intrinsic AKI when AKI was defined based on the creatinine criterion but again, there was no significant difference between no-AKI and transient AKI (p=0.49) when AKI was defined according to the diuresis criterion only (graphic illustration similar to figure 3; not shown).

Urinary NGAL levels and fractional excretion of urea in patients with no AKI vs transient vs intrinsic AKI, and this separately for those with a fractional sodium excretion below or above 0.36% are depicted in figure 4A and 4B respectively. Median urinary NGAL was higher in the overall group in those with high (>0.36%) versus low (<0.36%) fractional sodium excretion (median 1005 vs 314 µg/g creatininine respectively, p 0.025). In those with high fractional sodium excretion (>0.36%), urinary NGAL was higher in those with intrinsic AKI vs in those with transient AKI (median 4146 vs 1544 µg/g creatinine, p= 0.001). In those with a low fractional sodium excretion <0.36%, the difference was not significant (917 vs 286 µg/g creatinine, p=0.29) (figure 4A). Median fractional excretion of urea was higher (39.6% vs 28.6%, p=0.02) in the overall group in those with high (>0.36%) versus low (<0.36%) fractional sodium excretion. In those with high fractional sodium excretion (>0.36%), fractional urea excretion was lower in those with intrinsic AKI vs those with transient AKI (28,8% vs 38,7%, p 0.04). A RoC analysis of fractional urea excretion in patients with a fractional sodium excretion >0.36 yielded an AUC of 0.76 for discrimination between transient and intrinsic AKI. In those with a low fractional sodium excretion <0.36, the difference was not significant (21,6% vs 27,6%, p=0.49) (figure 4B)

The median volume of HES administered in no-AKI vs transient AKI based on urinary output was 0.5L vs 0.53L (p=0.15). Based on the creatinine criterion this was 0.5L and 0.5L in no-AKI vs transient AKI (p=0.64).

Figure 4: uNGAL(µg/g creatinine) and FEUrea(%) according to FENa (either < 0.36 or > 0.36%) in patients with noAKI, transient AKI and intrinsic AKI respectively.



Legend Figure 4: A: For patients with FENa <0.36%, uNGAL(µg/g creatinine) levels were not significantly different between no-AKI, transient AKI and intrinsic AKI. For patients with FENa >0.36%, uNGAL levels were significantly different between transient AKI and intrinsic AKI (p 0.001). B: For patients with FENa <0.36%, there was no significant difference in fractional excretion of urea between no-AKI, transient AKI and intrinsic AKI. For patients with FENa >0.36% there was a significant difference between patients with transient AKI and intrinsic AKI (p 0.04)

5.5 Discussion

When urinary output was used on top of serum creatinine in this cohort of sepsis patients to define AKI, more cases of AKI were detected as compared to when AKI was only based on the creatinine criterion. More importantly, transient AKI based on the RIFLE urinary output criterion was not associated with tubular damage as assessed by urinary NGAL, whereas there was tubular damage when diagnosis of transient AKI was based on the RIFLE creatinine criterion. Our data indicate that in patients with incipient sepsis, FENa is far below the proposed cut-off of 1%, which might explain the poor performance of the FENa<1% criterion. Unexpectedly, a persisting very low fractional urea excretion <31.5% was associated with intrinsic rather than transient AKI. A combined interpretation of high fractional sodium and urea excretion yielded a high negative predictive value for intrinsic AKI (92%) and persisting oliguria (95%), whereas a high fractional sodium and low fractional urea excretion had a 54% positive predictive value for patients remaining oliguric or developing intrinsic AKI.

Progress in the field of Acute Kidney Injury has been hampered by lack of a uniform definition. In the last decade, several recommendations for a consensus definition have been proposed[13-15]. However, although all these definitions are based on both serum creatinine and urinary output criteria together, the latter parameter has been neglected in many studies. This is mainly because urinary output is mostly not available in administrative databases, or because people believe it is difficult to register urinary output. As a consequence, there is ongoing debate on the value of urinary output for defining AKI[18-23].

However, oliguria is also associated with hard outcomes such as mortality[24], so it is now stressed that also urinary output should be taken into account when classifying AKI[15]. In our cohort, prevalence of the diagnosis of AKI decreased from 64.5 to 43% when urinary output was not taken into account. Whereas patients with transient AKI based on urinary output had similar levels of urinary NGAL production as patients without AKI, patients with transient AKI based on creatinine had levels of urinary NGAL production intermediate between no AKI and established AKI (figure 3), in line with the findings of Nejat et al, where only the creatinine criterion was used[8]. These results were confirmed when the fractional excretion of NGAL was used instead of urinary NGAL alone. These findings underline the concept that an increase in creatinine lags behind for early diagnosis of AKI, and that making a diagnosis based on urinary output might increase the potential for intervention at a stage when there is no tubular damage yet. Recently it was demonstrated in a post hoc analysis of the Chest study that fluid resuscitation with HES vs saline was more favorable when AKI was defined according to the diuresis criterion only, whereas saline was more favorable than HES when AKI was defined according to the creatinine criterion only[25], In our cohort the volume of HES administered was not different between no-AKI and transient AKI and between transient AKI based on the diuresis criterion vs transient AKI based on the creatinine criterion.

Recently, it was demonstrated that assessment of urinary output can be easily and accurately achieved by registering it in 6-8 hour blocks[16]. This approach would make it possible to even monitor urinary output in patients without an indwelling bladder catheter, and maybe even outside the intensive care care unit, at the hospital ward. This would be of importance, as it has been demonstrated that the majority of (fatal) cases of AKI develop unnoticed on the ward, and are diagnosed with much delay.[26]

There is discussion on the discriminatory value of fractional sodium excretion for the diagnosis of transient AKI. Usually, a cut off of <1% is forwarded as diagnostic discriminant.[27-29] Our data indicate that this cut off might be far too high, at least in sepsis, as 75% of the patients in our cohort had FENa<1%, and 50% even had a value <0.36%. Bagshaw et al recently reported an admission FENa below 1% in 57% of septic patients, being not different from non-septic patients[30]. Darmon et al reported a median FENa on admission of 0.50%.[31] Using RoC curve analysis, the same authors also found that a fractional excretion of 0.58% had the best discriminative power, resulting in a positive and negative predictive power for persistent AKI of 0.71 and 0.47 respectively.[31] In our cohort we used the median, being 0.36%.

Our data highlight some other pitfalls for the interpretation of a high fractional sodium excretion. First, a high fractional excretion of sodium can also be a marker of tubular damage, due to active tubular secretion and reduced proximal tubular reabsoption. In our cohort, potentially in line with presumed tubular damage, we indeed found higher urinary NGAL levels in patients with a high vs low fractional sodium excretion, and this most expressed in those who develop intrinsic AKI. Interestingly, in our cohort, patients with transient AKI had only higher urinary NGAL levels when they also had high fractional excretion of sodium >0.36% (figure4A), suggesting that some patients with transient AKI indeed develop some (minor) degree of tubular damage, whereas others do not. This also underpins that an increase in fractional sodium excretion is not always a positive sign, but can also be an indicator of subclinical tubular damage, which further adds to the low discriminative power of fractional sodium excretion, even when assessed in consecutive samples.

Second, a high fractional excretion of sodium might be caused by interference with use of diuretics. In our cohort, only a minority of patients were treated with diuretics, and those were equally distributed between AKI and no AKI. De Witte et al found much higher values of fractional sodium excretion in their cohort, but only a minority of their patients had sepsis, whereas 60% were treated with diuretics.[32] Because of these two reasons, a high FENa can thus not be interpreted by itself. Third, although the fractional excretion of sodium mainly decreases due to reduced urinary output and stimulation of sodium reabsorption, it can be speculated that fractional excretion of sodium also decreases when glomerular filtration decreases, e.g. because of tubular obstruction. Therefore, although in our cohort a very low fractional sodium excretion below <0.36% yielded a negative predictive value for intrinsic AKI of 87.3%, it is probably not a strong discriminative parameter, as was also suggested by Darmon et al[31].

Surprisingly, and against our initial hypothesis, a low (<31.5 %) fractional urea excretion at 4 hours was associated with intrinsic rather than with transient AKI, especially when it was persistent over time, whereas a value higher than 31.5% had a very high negative predictive value for intrinsic AKI. In patients with a fractional sodium excretion above 0.36%, the discriminatory value of fractional urea excretion was even more pronounced (figure 4B).

Darmon et al reported a FEUrea of 39% in the no AKI vs 41% in the transient AKI vs 32% in the intrinsic AKI group, and concluded that FEUrea had a low value for discriminating intrinsic AKI.[31] They based their analysis on admission FEUrea, whereas we used the value 4 hours after admission, when some attempts to restore low glomerular filtration pressure (e.g. by a fluid challenge or start of vasopressors) have already been installed. However, data based on FEUrea and FENa at admission showed comparable results, though less impressive (data not shown). Our findings are in line with those of De Witte et al, who also found a

reasonable value of FEurea<40% as a parameter to discriminate transient from intrinsic AKI.[32] As a low fractional excretion of urea apparently represents a low glomerular filtration, we hypothesized that a combination of fractional sodium and urea excretion would yield the most optimal prediction of patients with a substantial chance to respond positively to attempts to restore glomerular filtration. Indeed, in our cohort, a combination of a high fractional sodium and urea excretion yielded a 95% probability of restoring diuresis, and the AKI being transient rather than intrinsic, whereas a persistently low fractional urea excretion in combination with a high fractional sodium excretion was suggestive for intrinsic AKI.

There is debate on the discriminatory value of NGAL to predict or diagnose AKI in clinical conditions, as there is much overlap in NGAL levels between patients with vs. without AKI.[33] NGAL is a 25 kD molecule, that is filtered into the primary urine at the glomerular level. In sepsis, serum levels of NGAL increase exponentially[34;35], even in the absence of AKI[36], which can as such result in increased urinary levels as well[37]. Once filtered, NGAL is reabsorbed by the tubular epithelium through the megalin receptor and this through competitive binding[38].

Although a recent study indicated that urinary NGAL stems from local production in the thick ascending limb of the loop of Henle when stress factors are applied, there is also evidence that urinary NGAL can derive from the systemic circulation[37;39]. This indicates that the presence of NGAL in the urine in sepsis patients cannot automatically be considered as a marker of tubular damage per se. In addition, also some patients with transient AKI might have some degree of tubular damage, which might explain the relatively low value of NGAL to discriminate transient from intrinsic AKI in this cohort of septic patients.

A strength of this study is that it describes one of the largest cohorts of sepsis patients in detail for different patho-physiologic aspects of AKI, such as urinary and serum biomarkers, and urinary output. A limitation on the other hand is that it is observational and hence no causal assumptions can be made.

5.6 Conclusion

Urinary output is an early and sensitive marker of AKI, which might incite intervention before tubular damage has occurred. Low fractional excretion of sodium (<1%) and urea (<35%) is very frequent in septic patients and it might be necessary to define lower discriminatory cut-off values as an indication of maximal tubular reabsorption and thus intact tubular function as the ones used at present. A combination of high fractional excretion of sodium and urea has a high negative predictive value for intrinsic AKI, whereas a high fractional sodium excretion and a low fractional urea excretion are associated with intrinsic AKI in 54% of cases.

5.7 Reference List

1. Bellomo R, Bagshaw S, Langenberg C, Ronco C. Pre-renal azotemia: a flawed paradigm in critically ill septic patients? *Contrib Nephrol* 2007; 156: 1-9

2. Bellomo R, Wan L, Langenberg C, May C. Septic acute kidney injury: new concepts. *Nephron Exp Nephrol* 2008; 109: e95-100

3. Schrier RW, Wang W. Acute Renal Failure and Sepsis. *N Engl J Med* 2004; 351: 159-169

4. Prowle JR, Ishikawa K, May CN, Bellomo R. Renal blood flow during acute renal failure in man. *Blood Purif* 2009; 28: 216-225

5. Prowle JR, Molan MP, Hornsey E, Bellomo R. Measurement of renal blood flow by phase-contrast magnetic resonance imaging during septic acute kidney injury: a pilot investigation. *Critical Care Medicine 40 (6):1768 -76 ,* 2012;

6. Wan L, Bagshaw SM, Langenberg C, Saotome T, May C, Bellomo R. Pathophysiology of septic acute kidney injury: what do we really know? *Crit Care Med* 2008; 36: S198-S203

7. Langenberg C, Wan L, Egi M, May CN, Bellomo R. Renal blood flow in experimental septic acute renal failure. *Kidney International* 2006; 69: 1996-2002

8. Nejat M, Pickering JW, Devarajan P *et al*. Some biomarkers of acute kidney injury are increased in pre-renal acute injury. *Kidney Int* 2012; 81: 1254-1262

9. Doi K, Katagiri D, Negishi K *et al*. Mild elevation of urinary biomarkers in prerenal acute kidney injury. *Kidney Int* 2012; 82: 1114-1120

10. Bouchard J, Soroko SB, Chertow GM *et al*. Fluid accumulation, survival and recovery of kidney function in critically ill patients with acute kidney injury. *Kidney Int* 2009; 76: 422-427

11. Schrier RW. Fluid administration in critically ill patients with acute kidney injury. *Clin J Am Soc Nephrol* 2010; 5: 733-739

12. Kidney disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury . *Kidney Int Suppl* 2012; 2: 1-138

13. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; 8: R204-R212

14. Mehta RL, Kellum JA, Shah SV *et al*. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 2007; 11: R31

15. Fliser D, Laville M, Covic A *et al*. A European Renal Best Practice (ERBP) position statement on the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines on acute kidney injury: part 1: definitions, conservative management and contrast-induced nephropathy. *Nephrol Dial Transplant* 2012; 27: 4263-4272

16. Macedo E, Malhotra R, Claure-Del GR, Fedullo P, Mehta RL. Defining urine output criterion for acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2011; 26: 509-515

17. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20: 864-874

18. Bivet FG. Nonuse of RIFLE classification urine output criteria: biases for acute kidney injury biomarkers performance assessment? *Crit Care Med* 2012; 40: 1692-1693

19. Cruz DN, Bolgan I, Perazella MA *et al*. North East Italian Prospective Hospital Renal Outcome Survey on Acute Kidney Injury (NEiPHROS-AKI): targeting the problem with the RIFLE Criteria. *Clin J Am Soc Nephrol* 2007; 2: 418-425

20. Joannidis M. Classification of acute kidney injury: are we there yet? *Intensive Care Med* 2007; 33: 572-574

21. Mandelbaum T, Scott DJ, Lee J *et al*. Outcome of critically ill patients with acute kidney injury using the Acute Kidney Injury Network criteria. *Crit Care Med* 2011; 39: 2659-2664

22. Prowle JR, Liu YL, Licari E *et al*. Oliguria as predictive biomarker of acute kidney injury in critically ill patients. *Crit Care* 2011; 15: R172

23. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. *Kidney Int* 2008; 73: 538-546

24. Macedo E, Malhotra R, Bouchard J, Wynn SK, Mehta RL. Oliguria is an early predictor of higher mortality in critically ill patients. *Kidney Int* 2011; 80: 760-767

25. Myburgh JA, Finfer S, Bellomo R *et al*. Hydroxyethyl starch or saline for fluid resuscitation in intensive care. *N Engl J Med* 2012; 367: 1901-1911

26. MacLeod A. NCEPOD report on acute kidney injury-must do better. *Lancet* 2009; 374: 1405-1406

27. Bagshaw SM, Langenberg C, Bellomo R. Urinary biochemistry and microscopy in septic acute renal failure: a systematic review. *Am J Kidney Dis* 2006; 48: 695-705

28. Carvounis CP, Nisar S, Guro-Razuman S. Significance of the fractional excretion of urea in the differential diagnosis of acute renal failure. *Kidney International* 2002; 62: 2223-2229

29. Pepin MN, Bouchard J, Legault L, Ethier J. Diagnostic performance of fractional excretion of urea and fractional excretion of sodium in the evaluations of patients with acute kidney injury with or without diuretic treatment. *American Journal of Kidney Diseases* 2007; 50: 566-573

30. Bagshaw SM, Haase M, Haase-Fielitz A, Bennett M, Devarajan P, Bellomo R. A prospective evaluation of urine microscopy in septic and non-septic acute kidney injury. *Nephrol Dial Transplant* 2012; 27: 582-588

31. Darmon M, Vincent F, Dellamonica J *et al*. Diagnostic performance of fractional excretion of urea in the evaluation of critically ill patients with acute kidney injury: a multicenter cohort study. *Crit Care* 2011; 15: R178

32. Dewitte A, Biais M, Petit L *et al*. Fractional excretion of urea as a diagnostic index in acute kidney injury in intensive care patients. *J Crit Care* 2012; 27: 505-510

33. Vanmassenhove J, Vanholder R, Nagler E, Van BW. Urinary and serum biomarkers for the diagnosis of acute kidney injury: an in-depth review of the literature. *Nephrol Dial Transplant* 2013; 28: 254-273

34. Bagshaw SM, Bennett M, Haase M *et al*. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. *Intensive Care Med* 2010; 36: 452-461

35. Wheeler DS, Devarajan P, Ma Q *et al*. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. *Crit Care Med* 2008; 36: 1297-1303

36. Martensson J, Bell M, Oldner A, Xu S, Venge P, Martling CR. Neutrophil gelatinase-associated lipocalin in adult septic patients with and without acute kidney injury. *Intensive Care Medicine* 2010; 36: 1333-1340

37. Pedersen SS, Keller AK, Rehling M, Birn H, Jespersen B. NGAL excretion is higher from the healthy side than from the injured side in unilateral acute kidney injury. *Scand J Clin Lab Invest* 2012; 72: 510-512

38. Hvidberg V, Jacobsen C, Strong RK, Cowland JB, Moestrup SK, Borregaard N. The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin with high affinity and mediates its cellular uptake. *FEBS Lett* 2005; 579: 773-777

39. Paragas N, Qiu A, Zhang Q *et al*. The Ngal reporter mouse detects the response of the kidney to injury in real time. *Nat Med* 2011; 17: 216-222

CHAPTER 6: AKI IN EARLY SEPSIS IS A CONTINUUM FROM TRANSIENT AKI WITHOUT, OVER TRANSIENT AKI WITH MINOR TUBULAR DAMAGE TO INTRINSIC AKI WITH SEVERE TUBULAR DAMAGE.

Vanmassenhove J.[1], Glorieux G.[1], Hoste E.[2,3], Dhondt A.[1], Vanholder R.[1], Van Biesen W.[1]

[1]Renal Division, Ghent University Hospital

[2]Intensive Care Unit, Ghent University Hospital, [3]Senior Clinical Investigator – Research Foundation Flanders

Int Urol Nephrol. 2014 Oct;46(10):2003-8

6.1 Abstract

Purpose: The pathophysiology of septic Acute Kidney Injury (AKI) is incompletely understood and there is controversy on the role of renal hypoperfusion in early sepsis. We hypothesized that renal hypoperfusion plays a role in early sepsis and that there is a continuum between transient AKI without tubular damage, transient AKI with minor tubular damage and intrinsic AKI.

Methods: 107 consecutive patients with sepsis were included. Fractional excretion of sodium (FENa), urinary and serum Neutrophil gelatinase-associated Lipocalin (NGAL) were measured at admission(T0) and 4 hours(T4) and 24 hours later(T24). Patients were classified according to FENa quartiles. Transient and intrinsic AKI were respectively defined as AKI that did or did not recover to no AKI in the following 5 days.

Results: 57 developed transient AKI, 22 intrinsic AKI, and 28 did not have AKI. Of the 10 patients with transient AKI classified in the two lowest FENa quartiles (FENa<0.36%) and without signs of local tubular damage, 7 still did not show signs of tubular damage 24h later. Also, 50% of patients with intrinsic AKI classified in the same FENa quartiles, did not show signs of local tubular damage at admission but did so 24 hours later.

Conclusions: There is a continuum between transient AKI without tubular damage, transient AKI with minor tubular damage and intrinsic AKI in sepsis. Renal hypoperfusion seems to be the instigator for development of AKI in the majority of patients with early sepsis. Other mechanisms in some patients cannot be excluded.

6.2 Introduction

Septic Acute Kidney Injury (AKI) is associated with worse outcome compared to non-septic AKI and can be regarded as a distinct clinical entity.[1] The lack of significant progress in this field is partly related to an incomplete understanding of the pathophysiology of septic AKI.[2] The role of renal hypoperfusion in the pathogenesis of septic AKI is controversial and there is increasing evidence that inflammatory cascades and oxidative stress with microcirculatory changes play an important role as well. [3-6]

In recent years the idea has been put forward that transient acute kidney injury is also associated with a certain degree of tubular damage and is thus not merely a physiological response to a decrease in glomerular capillary perfusion. These assumptions are based on findings that showed an increase in biomarkers for diagnosis of tubular damage, in patients classified as having transient acute kidney injury. This has also led to the generation of a new subgroup of AKI, namely subclinical AKI, defined as a rise in biomarker level without a rise in serum creatinine or a decrease in urinary output.[7]

We hypothesized that renal hypoperfusion in early sepsis is an important underlying mechanism in the pathophysiology of septic AKI and that there is a continuum between transient AKI without tubular damage, transient AKI with minor tubular damage and intrinsic AKI.

6.3 Material and methods

107 consecutive patients admitted with sepsis, severe sepsis or septic shock were prospectively included at ICU in a tertiary university hospital between 12/01/2010 and 05/09/2010. The study was approved by the ethical committee of the Ghent University Hospital. Written informed consent was obtained from the patient or their next of kin. Sepsis, severe sepsis or septic shock were defined according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference guidelines.[8] Briefly, sepsis was defined as two or more of the following conditions being present as a result of infection: 1) temperature > 38° or < 36°, 2) heart rate > 90 beats/min, 3) respiratory rate > 20 breaths/min or PaCO2 < 32 mmHg (<4,3 kPa) or 4) white blood cell count > 12000 cells/mm^3 or < 4000 cells/mm^3 , or > 10% immature (band) forms. Severe sepsis was defined as sepsis associated with organ dysfunction, hypoperfusion or hypotension. Septic shock was defined as sepsis with hypotension despite adequate fluid resuscitation or vasopressor need. Exclusion criteria were 1) a history of transplantation, 2) ICU stay less than 24 hours, 3) patients treated with chronic hemodialysis, 4) age< 17 years and 5) obstructive Acute Kidney Injury.

The urinary output criterion was based on 6 hour blocks, as described by Macedo et al. [9] We defined AKI based on the worst of either creatinine or urinary output criteria according to RIFLE.[10] Baseline creatinine was based on the most recent value before admission or was estimated with the MDRD equation if the latter was not available. [10]

Transient AKI was defined as RIFLE R, I or F that improved to "no AKI" in the following five days, whereas "intrinsic AKI" was defined as patients with RIFLE R, I or F who did not evolve to no AKI in the following five days.

Fluid management and decision making for need of RRT was done by intensivists, blinded to the study, according to protocols applied in the hospital where the study was conducted.

Urine and blood samples were collected at the moment of admission (T0), four hours later (T4) and 24 hours later (T24). Blood samples were centrifuged within 20 minutes after collection at 1500g for 10 minutes, and serum was aliquoted and stored at -80°C for later batch analysis. Urine was collected in a sterile way and centrifuged at 500g for 10 minutes, urine samples were aliquoted and stored at -80°C for later batch analysis.

Fractional excretion of sodium was calculated according to the formula $(U_x x S_{crea})/(U_{crea} x S_x) x 100$ with x=sodium. Patients were classified according to FENa quartiles.

Serum and Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) were measured using an ELISA kit (Bioporto^R Diagnostics Denmark).

Since we previously found an important correlation between serum NGAL and urinary NGAL (unpublished data) we assessed the origin of urinary NGAL e.i. either serum NGAL after systemic generation, reduced tubular reabsorption of filtered NGAL or local NGAL release at distal tubular level, by using two parameters, namely "delta NGAL" and "diff NGAL". We defined "delta NGAL" as the urinary value minus the serum value, both expressed in ng/ml. Using delta NGAL might not be an exact numerical reflection of the final result of this complex process but one can easily appreciate that in case of an intact tubule, filtered NGAL can be integrally reabsorbed, and no local production of NGAL occurs, resulting in a net negative delta NGAL.[11;12] We also defined "diff NGAL" as the difference in urinary NGAL

between time points T4 and T0 and between time points T24 and T4. The urinary NGAL levels used to calculate diff NGAL were normalized for urinary creatinine, in order to correct for urinary flow. Since we were interested in parameters that could indicate tubular integrity, we defined both a deltaNGAL level ≤0 and a diffNGAL level ≤0 as absence of local NGAL production and thus absence of tubular damage, as the amount of NGAL entering the tubular system could not be retrieved down the road. If delta NGAL and diffNGAL were > 0 we considered that local NGAL production, and thus tubular damage could not be ruled out.

Results are reported as medians and interquartile ranges (IQR) for continuous variables, unless otherwise specified. Discrete variables are reported as numbers and/or percentages. All statistical analyses were performed using SPSS® 19. All consecutive patients were included, irrespective of their course or duration of stay at ICU.

6.4 Results

For demographics of the study cohort, including 107 patients with sepsis, severe sepsis or septic shock, we refer to a previously published paper.[13] In brief, 3.7% of patients had sepsis and 35.5% and 60.7% had severe sepsis and septic shock respectively. Also, 28(26.2%) were classified as having no-AKI, 57(53.3%) and 22(20.6%) as having transient and intrinsic AKI respectively[13]. The evolution of the serum creatinine values in patients with no-AKI, transient AKI and intrinsic AKI are described in table 1. A history of arterial hypertension was present in 41.3% of patients and 18.3%, 9.2%, 6.4% and 14.7% had a history of diabetes, stroke, peripheral arterial disease or coronary artery disease respectively. 10.1 and 15.6% suffered from heart failure or cardiac arythmia respectively. Cirrhosis and Chronic Obstructive Pulmonary Disease (COPD) were present in 5.5% and 11% respectively. 37% were either currenly or in the past treated for an oncological disease. The focus of infection was predominantly 'respiratory' in 40.2% of patients, followed by 'abdominal' (30.8%), 'urinary' (6.5%), 'endocarditis' (4.7%), 'catheter related' (3.7%) and 'neurological' (2.8%). The focus of infection was related to other causes or unknown in 12 patients (11.2%).

Table 1: Evolution of serum creatinine (median/IQR) over the first three days after ICU admission in no-AKI vs transient AKI vs intrinsic AKI.

	T0	T4	T24	T48	T72
No-AKI	0.67(0.35)	0.62(0.41)	0.66(0.40)	0.60(0.40)	0.59(0.34)
Transient AKI	1.0(0.75)	0.94(0.71)	0.88(0.57)	0.75(0.45)	0.71(0.45)
Intrinsic AKI	1.45(1.61)	1.84(1.22)	1.92(1.38)	1.77(1.53)	1.90(1.68)

Legend: T0=sCr at study inclusion, T4= sCr 4 hours after study inclusion, T24=first sCr value at day 1, T48= first sCr value at day 2, T72=first sCr value at day 3

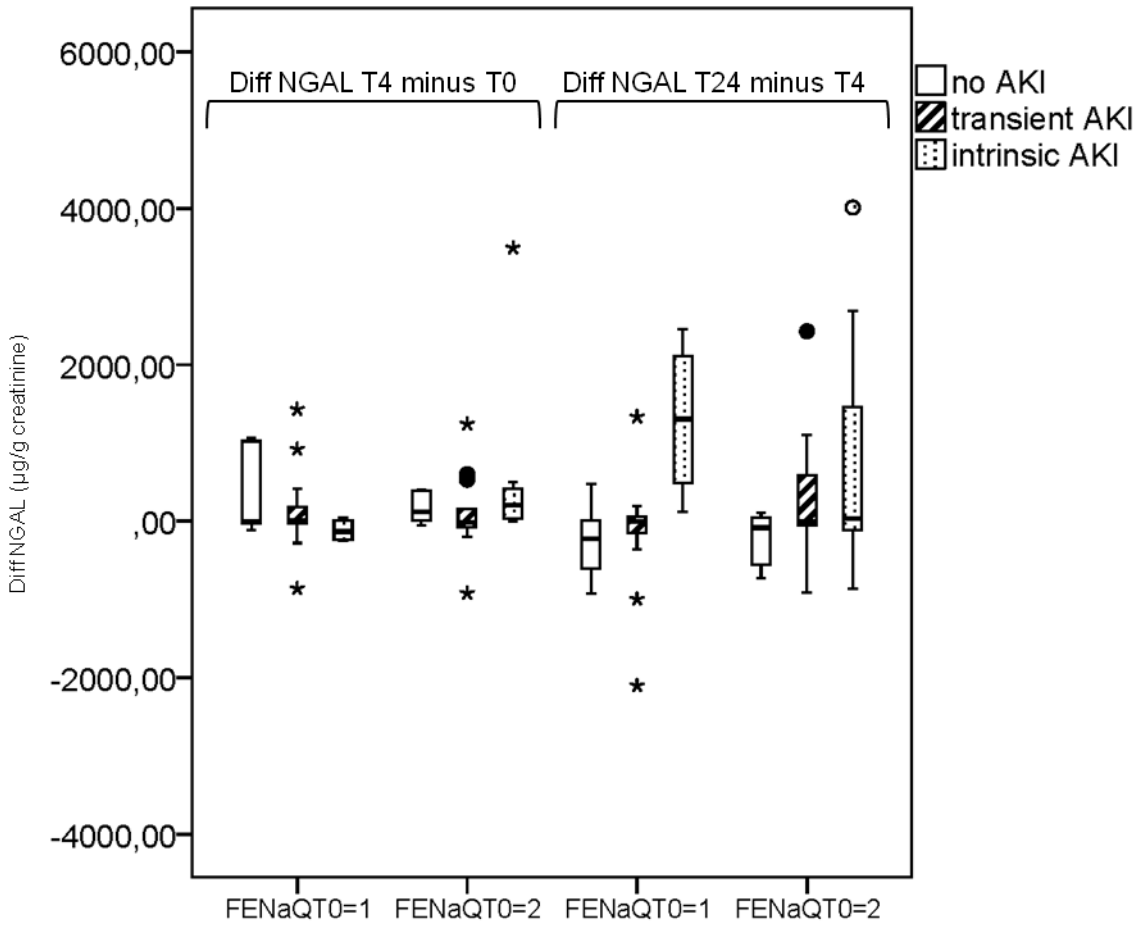
We previously demonstrated that FENa<1% is prevalent in early sepsis.[13] Twelve out of 28 (42.9%), 29/57 (50.9%) and 12/22 (54.5%) of patients classified as having no-AKI, transient AKI and intrinsic AKI respectively, were classified in the lowest two sodium quartiles (FENa<0.36%) at admission(T0).

At admission, 7 of the 10 patients who were classified as having transient AKI with a FENa less than 0.36% (FENa quartile 1 and FENa quartile 2) and both DeltaNGAL and DiffNGAL ≤0,

indicating tubular integrity, still did not show signs of tubular damage 24 hours later illustrating that some patients classified as having transient AKI do not have signs of tubular damage in the first 24 hours after admission (figure 1).

Fifty % of patients classified as having intrinsic AKI with a FENA less than 0.36% and both DeltaNGAL and DiffNGAL≤0 at admission, did show an increase in FENa and positive values for both diffNGAL and deltaNGAL 24 hours later (figure 1), suggesting that intrinsic AKI might start of as renal hypoperfusion before displaying signs of progressive tubular damage.

Figure 1: Tubular integrity defined as diffNGAL≤0 in patients with no-AKI, transient AKI and intrinsic AKI, classified in the lower sodium quartiles (FENa<0.36%).

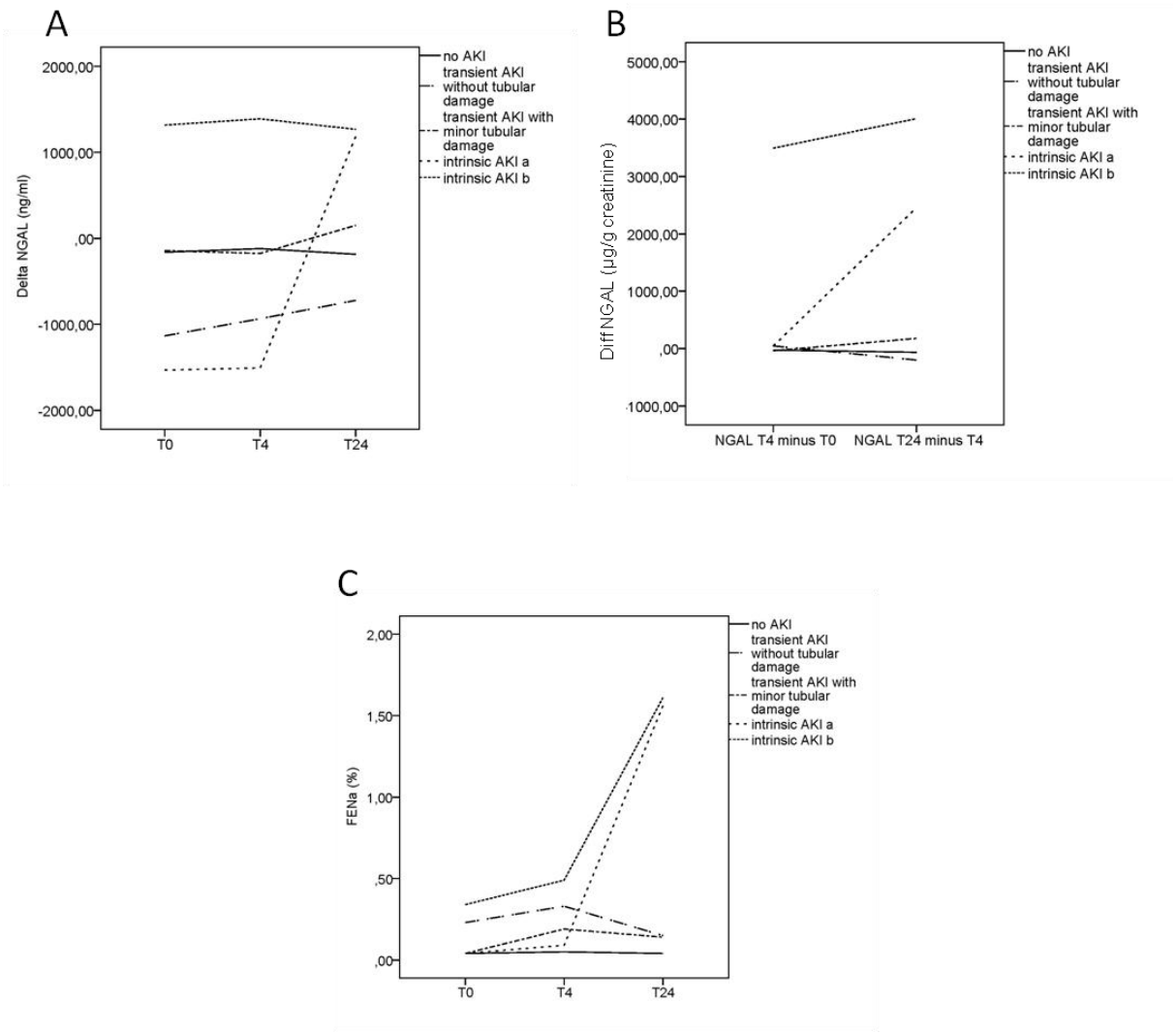


Legend Figure 1: A substantial part of patients with transient AKI, classified in the lower sodium quartiles at T0(FENaQT0=1 or 2), indicating maximal tubular sodium reabsorption, do not show local NGAL production at admission and still don't have signs of local NGAL production, as illustrated by diffNGAL≤0, 24 hours later. Some patients with intrinsic AKI do not show signs of tubular damage at T0, but do so 24 hours later. (Similar results for deltaNGAL, figure not shown)

To further illustrate that the continuum of "no AKI", "transient AKI without tubular damage", "transient AKI with minor tubular damage", "evolution to intrinsic AKI (intrinsic a)" and "intrinsic AKI from the start (intrinsic b)", is a reality in patients with sepsis, a graphic illustration of DeltaNGAL, diffNGAL and FENa, at the different time points (T0,T4 and T24) in five representative patients classified in one of the groups mentioned above is presented in

figure 2. Delta NGAL levels and diffNGAL ≤0 indicate absence of local NGAL production in patients with no-AKI, and transient AKI without tubular damage. On the other hand, a patient with 'evolution to intrinsic AKI', shows a gradual increase in local NGAL production over time together with an increase in FENa (intrinsic a). A patient with 'intrinsic AKI from the start', shows increased levels of local NGAL production from the beginning (intrinsic b) (Figure 2).

Figure 2: DeltaNGAL, diffNGAL and FENA in patients with no-AKI, transient AKI without tubular damage, transient AKI with minor tubular damage, intrinsic AKI type a and intrinsic AKI type b, at the different time points.



Legend Figure 2: (a) Delta NGAL levels indicate absence of local NGAL production in patients classified as having no-AKI and transient AKI without tubular damage. Patients with intrinsic AKI type a (evolution from renal hypoperfusion without tubular damage to intrinsic AKI), show a gradual increase in Delta NGAL over time. The patient classified as having intrinsic AKI type b shows increased levels of delta NGAL from the start. Transient AKI with minor tubular damage is associated with levels of DeltaNGAL that are lower than in intrinsic AKI. (b) DiffNGAL levels indicate absence of local NGAL production in patients classified as no-AKI and trannsient AKI without tubular damage. Patients with intrinsic AKI type a, show a gradual increase in Diff NGAL level over time. The patient classified as intrinsic type b shows increased levels of diffNGAL from the start. Transient AKI with minor tubular damage is associated with levels of Diff NGAL that are lower than in intrinsic AKI. (c) FENa remains below 1% in all patients at all time points except for Intrinsic AKI type a and intrinsic AKI type b, at time point T24.

6.5 Discussion

In a cohort of 107 septic patients we showed that there is a continuum between transient AKI without tubular damage, transient AKI with minor tubular damage and intrinsic AKI. Although there is controversy on the role of renal hypoperfusion in the pathophysiology of septic AKI, renal hypoperfusion seems to be the instigator of AKI in the majority of patients in this sepsis cohort.

In this cohort, a substantial part of patients classified as having transient AKI did not show signs of tubular damage at admission, as suggested by a very low FENa (FENa quartile 1 or 2 = FENa <0.36%) and a deltaNGAL and diffNGAL ≤0. Many of them still did not even show signs of tubular damage 24 hours later. This underlines the hypothesis that persisting prerenal azotemia without tubular damage can exist in early sepsis. In recent literature there has been discussion on this topic [14] and the reliance on urinary biomarkers such as NGAL as markers for tubular damage has led to the creation of a new entity, called subclinical AKI.[7] The latter is defined as a condition associated with an increase in biomarker level without fulfilling the classic RIFLE criteria based on serum creatinine and urinary output.[7] Also, in view of the search for new 'troponin-like' biomarkers there are several reports on the need for defining a renal angina syndrome equivalent. The latter is currently based on clinical factors such as serum creatinine and oliguria but authors hint that in the future the diagnosis of renal angina could be based on new urinary biomarkers such as NGAL or KIM-1.[15]

The consequences of incorrectly classifying patients as having AKI when they actually don't have AKI, can be that studies testing new drugs risk to be falsely negative, because they include patients with so called subclinical AKI in the placebo group.

The emergence of the term 'subclinical AKI' has also led to the dismissal of the concept of prerenal azotemia, defined as a physiological response to a decrease in renal perfusion without structural damage. It created the general impression that there is always a certain degree of tubular damage if there is a decrease in GFR and/or if urinary biomarkers are present.

Two recent studies are in favor of presence of structural damage in patients with so-called prerenal azotemia.[16;17] Doi et al suggested that new AKI biomarkers such as NGAL, LFABP, IL-18, NAG and albumin can detect mild renal tubular damage in prerenal acute kidney injury in a study including 337 critically ill patients and Nejat et al showed that biomarker concentrations significantly and progressively increased with the duration of AKI.[16;17]

Although the findings in both studies led the authors to conclude that prerenal azotemia is associated with a certain degree of tubular damage, some questions remain open. First, NGAL levels were not statistically different between prerenal and no-AKI in the study by Nejat et al[17] and in the volume depleted mice in the study by Doi et al.[16] Also in another recent study no increase in NGAL levels in volume depleted animals was demonstrated, which led authors to hypothesize that NGAL might be less sensitive to differentiate prerenal from no-AKI.[18] Although these results at least suggest that in genuine prerenal azotemia there is no increase in NGAL levels, still a lot of effort seems to be put in convincing the scientific world that a rise in biomarker level (f.e. NGAL) is always a sign of tubular damage, even in the absence of AKI diagnosis by classical criteria such as serum creatinine increase of oliguria.[19;20] A more plausible explanation is that test results indicating biomarker positivity,

can be falsely positive and thus that the presence of these biomarkers in the urine is not necessarily a sign of tubular damage.

Second, there are pitfalls in using these molecules as markers for tubular damage. These are related to the fact that results can be influenced by several non-renal conditions, such as inflammation.[21] This is corroborated by the fact that in the studies of Nejat et al and Doi et al also no-AKI patients showed increased biomarker levels.[16;17]

In our cohort, some patients who were classified as having intrinsic AKI did not show signs of tubular damage at the first time point, but did so 24 hours later. These findings suggest that septic AKI is a continuum between transient AKI without tubular damage (as evidenced by a very low fractional excretion of sodium and no signs of local tubular damage) over transient AKI with limited tubular damage, to full blown AKI with extensive tissue damage. As a consequence, restoring renal perfusion in the first hours of sepsis is a potentially effective way of preventing AKI.

However, some patients classified as having intrinsic AKI show signs of tubular damage from the beginning and thus other mechanisms besides renal hypoperfusion in the pathogenesis of early septic AKI cannot be excluded.

In the study of Nejat et al and Doi et al, prerenal AKI was defined as recovery of AKI within 48 hours and a FENa<1%, making it implicitly impossible to demonstrate that intrinsic AKI can start off as prerenal azotemia.[16;17]

A limitation of this observational study is that it describes a relatively small cohort of patients. However the study provides information on both FENa, serum NGAL and urinary NGAL at different time points during the first 24 hours after admission in septic patients. Since we were only interested in having strict markers for tubular integrity we used the lowest two FENa quartiles (FENa <0.36%) and both diffNGAL and deltaNGAL ≤ 0. We fully acknowledge that even more patients could show this continuum since a positive deltaNGAL or diffNGAL might arise even in the absence of tubular damage. However, a negative value almost certainly excludes the possibility of tubular damage. Since our goal was to illustrate that there is a continuum between transient AKI without tubular damage, transient AKI with minor tubular damage and intrinsic AKI, we preferred parameters for tubular integrity that potentially were too strict vs the opposite. This resulted in a high degree of certainty regarding the presence of tubular integrity when DeltaNGAL and diffNGAL were both ≤0. However, if one or both criteria were >0, tubular damage could not be ruled out.

We also acknowledge that fluid resuscitation before ICU admission could have influenced AKI diagnosis. However, this would have weakened rather than strengthened our findings.

6.6 Conclusion

There is a continuum between transient AKI without tubular damage, transient AKI with minor tubular damage and intrinsic AKI in sepsis. Renal hypoperfusion seems to be the instigator for development of AKI in the majority of patients with early sepsis. Other mechanisms in some patients cannot be excluded.

6.7 Reference List

1. Bagshaw SM, Uchino S, Bellomo R *et al*. Septic acute kidney injury in critically ill patients: clinical characteristics and outcomes. *Clinical Journal of The American Society of Nephrology: CJASN* 2007; 2: 431-439

2. Wan L, Bagshaw SM, Langenberg C, Saotome T, May C, Bellomo R. Pathophysiology of septic acute kidney injury: what do we really know? *Crit Care Med* 2008; 36: S198-S203

3. Matejovic M, Radermacher P, Joannidis M. Acute kidney injury in sepsis: is renal blood flow more than just an innocent bystander? *Intensive Care Medicine* 2007; 33: 1498-1500

4. Molitoris BA. Renal blood flow in sepsis: a complex issue. *Critical Care (London, England)* 2005; 9: 327-328

5. Wang Z, Holthoff JH, Seely KA *et al*. Development of oxidative stress in the peritubular capillary microenvironment mediates sepsis-induced renal microcirculatory failure and acute kidney injury. *American Journal of Pathology* 2012; 180: 505-516

6. Zarjou A, Agarwal A. Sepsis and acute kidney injury. [Review]. *Journal of the American Society of Nephrology* 2011; 22: 999-1006

7. Haase M, Kellum JA, Ronco C. Subclinical AKI--an emerging syndrome with important consequences. *Nat Rev Nephrol* 2012; 8: 735-739

8. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20: 864-874

9. Macedo E, Malhotra R, Claure-Del GR, Fedullo P, Mehta RL. Defining urine output criterion for acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2011; 26: 509-515

10. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; 8: R204-R212

11. Axelsson L, Bergenfeldt M, Ohlsson K. Studies of the release and turnover of a human neutrophil lipocalin. *Scand J Clin Lab Invest* 1995; 55: 577-588

12. Mori K, Lee HT, Rapoport D *et al*. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. *J Clin Invest* 2005; 115: 610-621

13. Vanmassenhove J, Glorieux G, Hoste E, Dhondt A, Vanholder R, Van BW. Urinary output and fractional excretion of sodium and urea as indicators of transient versus intrinsic acute kidney injury during early sepsis. *Crit Care* 2013; 17: R234

14. Bellomo R, Bagshaw S, Langenberg C, Ronco C. Pre-renal azotemia: a flawed paradigm in critically ill septic patients? *Contrib Nephrol* 2007; 156: 1-9

15. Goldstein SL, Chawla LS. Renal angina. *Clin J Am Soc Nephrol* 2010; 5: 943-949

16. Doi K, Katagiri D, Negishi K *et al*. Mild elevation of urinary biomarkers in prerenal acute kidney injury. *Kidney Int* 2012; 82: 1114-1120

17. Nejat M, Pickering JW, Devarajan P *et al*. Some biomarkers of acute kidney injury are increased in pre-renal acute injury. *Kidney Int* 2012; 81: 1254-1262

18. Paragas N, Qiu A, Zhang Q *et al*. The Ngal reporter mouse detects the response of the kidney to injury in real time. *Nat Med* 2011; 17: 216-222

19. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 2009; 54: 1012-1024

20. Haase M, Devarajan P, Haase-Fielitz A *et al*. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. *J Am Coll Cardiol* 2011; 57: 1752-1761

21. Giasson J, Li GH, Chen Y. Neutrophil gelatinase-associated lipocalin (NGAL) as a new biomarker for non--acute kidney injury (AKI) diseases. *Inflamm Allergy Drug Targets* 2011; 10: 272-282

CHAPTER 7:

DISCUSSION

7.1 Study Cohort

7.1.1 Demographic Data

We described a cohort of 195 prospectively included patients with sepsis, admitted to the Ghent University Hospital between 12/01/2010 and 27/03/2011. Patiënts who developed sepsis *during* their ICU (Intensive Care Unit) stay, were not considered for inclusion. Sepsis, severe sepsis and septic shock were defined according to the ACCP/SCCM criteria.[1] Exclusion criteria were 1) ICU stay less than 24 hours or withdrawal of therapy, 2) no bladder catheter, 3) patients treated with chronic hemodialysis, 4) patients with RRT need due to AKI *upon* ICU admission, 4) Age < 17 years, 5) a history of organ transplantation, 6) obstructive AKI and 7) no central line or arterial catheter. During the study period, 253 patients were considered for inclusion of whom 58 were excluded (18 for not having a bladder catheter, 13 because of RRT need upon ICU admission, 10 with a history of organ transplantation, 7 because of the decision to withdraw therapy, 5 for being treated with chronic dialysis, 3 who had an ICU stay < 24h, 1 with obstructive AKI and 1 who did not have an arterial or central venous line). NGAL measurements were performed in the first 107 patients included.

The demographic data of this cohort are depicted in Table 1.

Table 1: Demographics of the study cohort

Age(years, mean/sd)	**60.6(15.5)**
Gender(%Male)	61.4(15)
Sepsis Severity (n/%)	
Sepsis	9(5)
Severe Sepsis	63(32)
Septic Shock	123(63)
ICU Mortality (%)	
Sepsis	11
Severe Sepsis	10
Septic Shock	31
90 days Mortality (%)	
Sepsis	11
Severe Sepsis	16
Septic Shock	41
Reason for admission (%)	
Respiratory	42
Abdominal	29
Urosepsis/Urological	7
Endocarditis	4
Neurological	3
Catheter	2
Other/Unknown	14
APACHE II score first 24h after admission	23(9)
Need for ventilation during ICU stay (%)	54
Vasopressor need (%)	61
LOS ICU survivors (days)	6(9)
CKD (eGFR<60ml/min/1,73m2) (%)	15
Use of diuretics on the first day of admission (%)	14

7.1.2 Prevalence of AKI according to RIFLE

7.1.2.1 AKI and RIFLE

According to RIFLE, based on both the serum creatinine (sCr) and urinary output criteria, 131 patients (67%) developed AKI in the first 24 hours after admission. 201% were classified as RIFLE-R (Risk) vs 29% and 17% as RIFLE-I (Injury) and RIFLE-F (Failure), respectively (Table 2). Over the next four days after ICU admission, the number of patients diagnosed with AKI increased to 152(78%) of whom 23%, 31% and 25% were classified as RIFLE-R, RIFLE-I and RIFLE-F, respectively (Table 2). Thus the majority in our cohort of sepsis patients that fulfilled the criteria for AKI did so already in the first 24h after admission (Table 2).

Table 2: Prevalence of AKI according to RIFLE and RRT need

AKI according to RIFLE first 24h (n(%))	
RIFLE-Risk	**40(21)**
RIFLE-Injury	**57(29)**
RIFLE-Failure	**34(17)**
Worst RIFLE class during the first 5 days after admission (n/%)	
RIFLE-Risk	**44(23)**
RIFLE-Injury	**60(31)**
RIFLE-Failure	**48(25)**
RRT need (%)	27(14)

In a multicenter evaluation of the RIFLE criteria for AKI in critically ill patients, Bagshaw et al found that the prevalence of AKI on the first day of admission was 36.1% with 16.3%, 13.6% and 6.3% classified as RIFLE-R, RIFLE-I and RIFLE-F respectively.[2] A subgroup analysis demonstrated a higher prevalence of AKI in septic vs non-septic AKI (42.1% with 16.2%, 16.3% and 9.6% classified as RIFLE-R, RIFLE-I and RIFLE-F respectively).[3] However this was a retrospective study, urinary output criteria were modified and baseline sCr values were estimated, which might explain the difference in prevalence compared to our study.

In two other studies[4;5], AKI occurred in 11-16% of patients, however the AKI definition that was applied also differed from ours. Hoste et al[4] defined AKI as an abrupt increase of sCr to more than 2 mg/dl in a exclusively surgical ICU and Yegenaga et al[5] used the same sCr increase criterion and added an a urinary output criterion by defining oliguria as a urinary output ≤ 400ml/24h.

AKI prevalence was around 35% in two retrospective studies by Lopes et al and Ostermann et al in patients with sepsis and in the critically ill, respectively[6.7] In the study by Lopes et al[8], authors did not state whether both RIFLE criteria were used for defining AKI. Ostermann et al[7] used modified urinary output criteria and eGFR, the latter being unreliable in non-steady state conditions such as AKI. Oppert et al[9] also found a lower AKI prevalence than we did in a

prospective cross sectional one-day study including critically ill sepsis patients in 454 ICU's over 310 German hospitals. They defined AKI as a sCr increase above twice the upper limit or a urinary output < 0.5 ml/kg/h for at least 4h despite fluid resuscitation.[9]

Several of the above mentioned studies[4;5;9] did not include CKD patients which might also have influenced AKI prevalence because CKD patients tend to be more susceptible to develop AKI, although this remains controversial in sepsis patients.[10-12]

7.1.2.2 AKI and RRT

The prevalence of RRT need in critically ill and/or sepsis patients also varies and depends on the AKI severity of the patients included and the hospital and/or physician's practices for starting RRT. In the SOAP study, 7% of patients were treated with hemodialysis and 13% with hemofiltration within the sepsis subgroup.[13] In a study of Bagshaw et al[14], septic AKI resulted in RRT need in 8.2% (72/883) of AKI patients and in a study by Hoste et al[4], 70% of included ARF patients were treated with RRT, comparable to the results of the PICARD study where 64% of 618 critically ill patients with ARF were treated with RRT.[15]

In our cohort, 27(14%) of all included patients were treated with RRT. However 13 patients were excluded because they already had RRT need on the time of ICU admission. 10/110(9%) and 8/110(7%) of patients were treated with RRT although they were classified on the first day of admission as having no AKI according to the urinary output criterion only, or the sCr criterion only respectively (Figure 1). Patients classified as having no-AKI based on both criteria on the first day of admission received RRT in 5% (3/64) (Figure 1). Of the patients classified as having no-AKI on D0 *and* D4 based on either the urinary output criterion only, the sCr criterion only or both criteria, 5% (5/95) , 3% (3/100) and 2% (1/52) respectively, were treated with RRT (Figure 1). Two of the five patients who were treated with dialysis although they were not oliguric during ICU stay, only received dialysis on 1 occasion and for the following indications: high uremia and therefore anticipated problems for weaning (due to confusion of the patient) in one and lactic acidosis in another patient. A third patient who was not oliguric during ICU stay developed RRT need only at day 14 after ICU admission and was oligo-anuric at that time. Patients classified as RIFLE-F according to the urinary ouput criterion on the first day of admission received more frequently RRT compared to patients classified as RIFLE-F according to the sCr criterion (9/11(82%) vs 8/27(30%)). Patients with a worst RIFLE stage 'F' over 5 days according to the urinary output criterion only (n=19) were more frequently treated with RRT than when their worst RIFLE stage was RIFLE-F according to the serum creatinine criterion (n=42) only (79% vs 50% RRT need respectively) (Figure 1). These findings suggest that urinary output is a more specific criterion than sCr for prediction of RRT and put emphasis on the fact that both criteria (sCr and urinary output) should be included in the AKI definition.

7.1.2.3 Evolution of RIFLE

Eighty-one percent of patients classified as having no-AKI in the first day of ICU admission, according to both the sCr and the urinary output criterion, did not develop AKI during their ICU stay. The majority of patients classified as having RIFLE-R on the first day of admission, do not progress and are classified as no-AKI on D4 (37/40=93%) (Figure 1). Of the patients classified as RIFLE-I (n=57) or RIFLE-F (n=34), 30% (17/57) and 56% (19/34) are still classified as RIFLE-I or RIFLE-F on D4. Of those, 6% (1/17) and 68% (13/19) were treated with RRT. Only 7 of 57(12%) patients classified as RIFLE-I on D0 progressed to RIFLE-F on D4 (Figure 1).

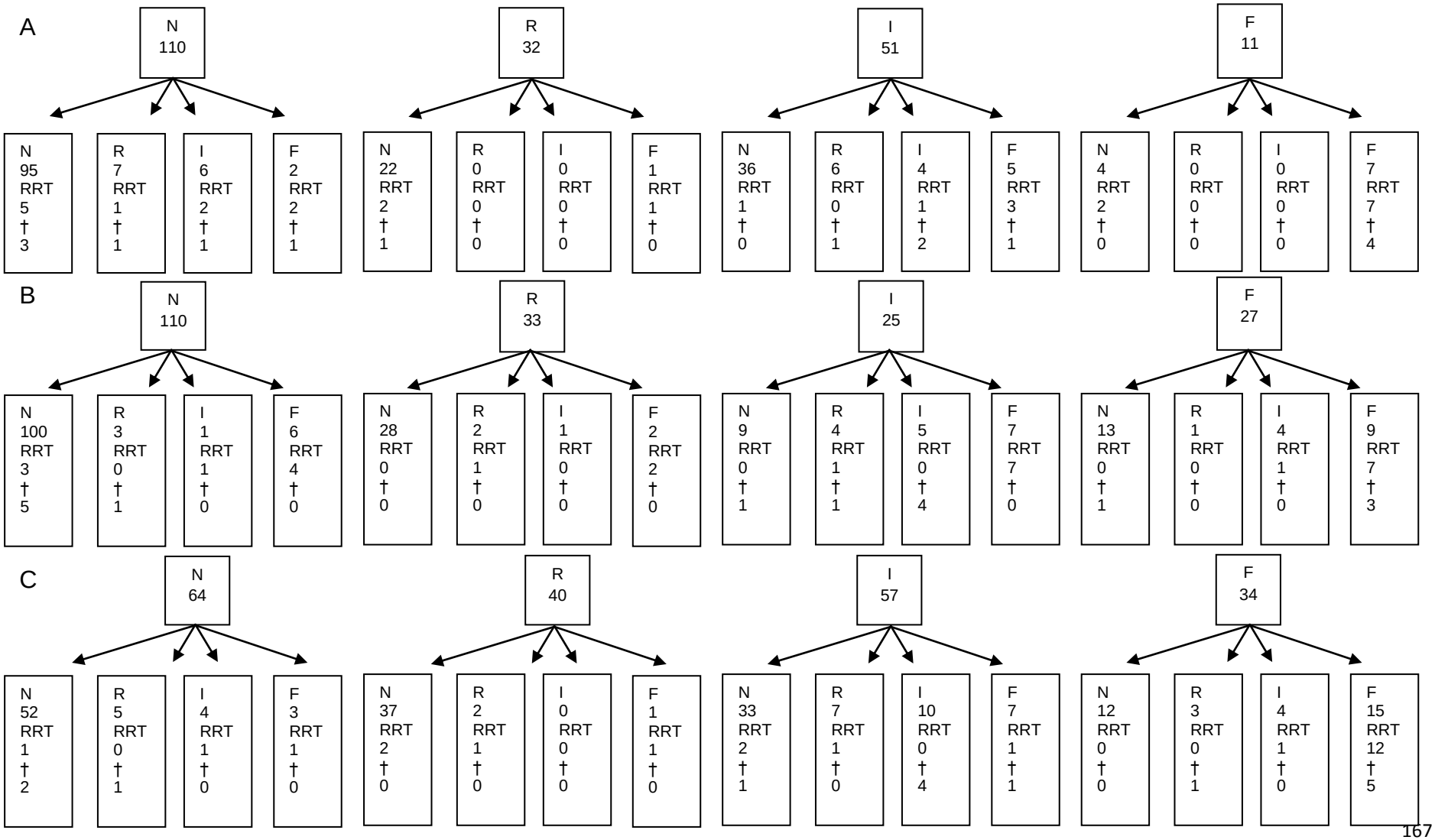
These results are not corroborated by a retrospective study of Hoste et al[16] who found that in a cohort of 5383 critically ill patients of whom 67.2% developed AKI during their ICU stay, 50% progressed from RIFLE-R on the first day of admission to RIFLE-I or RIFLE-F in the following days and more than one third of the patients with RIFLE-I progressed to RIFLE-F.

In their cohort, less than 1% of patients classified as having RIFLE-I and 14.2% of those classified as RIFLE-F, were treated with RRT vs 11 and 58% respectively, in our cohort.[16]

Altogether, epidemiological data on AKI prevalence and RRT need seem to differ widely, even within the same setting. This might be attributable to the lack of a uniform definition and/or a uniform application of the RIFLE criteria (different reference/baseline values and omitting or modifying the urinary output criteria)(see also chapter 7.2).

Figure 1: Evolution of RIFLE according to the urinary output criterion (A), the sCr criterion (B) or both criteria (C), over the first 5 days after ICU admission (RIFLE classification on day 5 versus RIFLE classification on the first day ICU admission).

(N=no AKI, R= Risk, I=Injury, F=Failure, RRT=treatment with RRT during ICU stay, †: patients who died during the first 5 days after ICU admission)



7.1.3 Outcome

We found high overall short and median term mortality rates in this cohort of sepsis patients with ICU, 90 days, 1 year and 2 years mortality rates of 23.1%, 31.3%, 43.6% and 50.3% respectively. In ICU survivors, 90 days, 1 year and 2 years mortality remained high with rates of 11.3%, 26.7% and 35.3% respectively. (Table 3)

Of the 27 patients treated with RRT, cumulative mortality was 55.6% during ICU stay. Eighty-three % of patients who were treated with RRT and survived ICU (n=10/12) survived up to 2 years. One patient treated with RRT died at three months and another patient died at year 1.

7.1.3.1 Sepsis and mortality

In a multicenter study of French ICU's including 546 patients with severe sepsis or septic shock, 30 day mortality rate was 35% and hospital mortality rate was 41.9%.[17] This French cohort differed from ours in age (mean age 65 vs 60.6 years) and in severity of illness with also less severily ill patients included in our study (patients with sepsis, severe sepsis or septic shock vs only severe sepsis and septic shock in the study by Brun-Buisson).[17] In the SOAP study, 3147 patients were included over a period of two weeks in 198 ICU's across 24 European countries. 37.4% had sepsis with an ICU mortality of 27% vs 14% in non-septic AKI.[13] There was a correlation between the ICU mortality rate for all patients and the sepsis rate in the various countries.[13] Also in the SOAP study patients had a higher mean age than in our study (again mean age 65 vs 60.6 years). Overall, several reports seem to suggest that mortality rates in sepsis are decreasing.[18;19] The total number of sepsis related deaths is increasing but the case fatality rate is decreasing.[20] There are several potential explanations for the decrease in case fatality rate. First, there has been a significant improvement in the treatment of patients with sepsis in the last decades and technical/surgical procedures and skills have improved. Second, there might be a greater awareness for diagnosis of sepsis patients among physicians with earlier referral to ICU which might impact outcome. Third, due to budgetary reasons physicians might be more strict in registering patients as having sepsis in order to increase reimbursement.

7.1.3.2 AKI according to RIFLE and mortality

Although it is generally accepted that AKI is an independent predictor of mortality[21-25], there is still controversy on the topic, even in the non-critically ill setting. In a population based study by Ali et al, there was no association between AKI and outcome.[26] Also, Wald et al reported a higher risk for RRT need but not mortality, in a large cohort of ICU survivors.[27] Lo et al reported a much higher (28-fold) risk increase for CKD 4-5 development than for mortality (2-fold) in patients with AKI vs no AKI.[28]

In our cohort of sepsis patients, we did not find an increase in mortality rates from no-AKI, over RIFLE-R and RIFLE-I to RIFLE-F, either defined according to RIFLE based on both criteria,

over the first 24 hours after admission or over the first 5 days after admission (Table 2 and Table 3). Defining AKI according to either the urinary output criterion only or the sCr criterion only, did not change these findings. Based on both criteria in the first 24h after admission, ICU mortality rates in no-AKI, RIFLE-R, RIFLE-I and RIFLE-F were 22%, 15%, 23% and 35% respectively and 21%, 18%, 18% and 35% respectively when AKI was defined over 5 days (Table 3). At 90 days, 1 year and 2 years findings were similar with an absence of gradual increase in no-AKI, RIFLE-R, RIFLE-I and RIFLE-F (Table 3).

Although in a systematic review, Ricci et al[29] concluded that there was a gradual increase in mortality with increasing stages of AKI, most of the included studies were retrospective and RIFLE criteria were being applied differently and often urinary output data were lacking.[29] Also, only 13/16 studies comparing mortality between RIFLE strata of severity reported on the mortality rate in the no-AKI group and only 1 paper, which was a letter, including exclusively sepsis patients.[30] It is also important to note that the RIFLE criteria were originally not intended as a tool for prediction of mortality.

Table 3: Mortality in the entire cohort (A) and in ICU survivors (B) and according to AKI status (RIFLE based on both criteria) and time span (AKI diagnosis over the first 24h vs over the first 5 days after ICU admission).

A

ICU Mortality(%)	23
No AKI$_{first24h}$	22
RIFLE$_{first24h}$-Risk	15
RIFLE$_{first24h}$-Injury	23
RIFLE$_{first24h}$-Failure	35
No AKI$_{first5days}$	21
RIFLE$_{first5days}$-Risk	18
RIFLE$_{first5days}$-Injury	18
RIFLE$_{first5days}$-Failure	35
90 days Mortality(%)	31
No AKI$_{first24h}$	31
RIFLE$_{first24h}$-Risk	23
RIFLE$_{first24h}$-Injury	32
RIFLE$_{first24h}$-Failure	41
No AKI$_{first5days}$	33
RIFLE$_{first5days}$-Risk	23
RIFLE$_{first5days}$-Injury	30
RIFLE$_{first5days}$-Failure	40
1-year Mortality(%)	44
No AKI$_{first24h}$	48
RIFLE$_{first24h}$-Risk	33
RIFLE$_{first24h}$-Injury	44
RIFLE$_{first24h}$-Failure	47
No AKI$_{first5days}$	44

RIFLE$_{first5days}$-Risk	36
RIFLE$_{first5days}$-Injury	43
RIFLE$_{first5days}$-Failure	50
2-years Mortality(%)	50
No AKI$_{first24h}$	56
RIFLE$_{first24h}$-Risk	43
RIFLE$_{first24h}$-Injury	51
RIFLE$_{first24h}$-Failure	47
No AKI$_{first5days}$	51
RIFLE$_{first5days}$-Risk	48
RIFLE$_{first5days}$-Injury	52
RIFLE$_{first5days}$-Failure	50

B

90 days Mortality(%)	11
No AKI$_{first24h}$	12
RIFLE$_{first24h}$-Risk	12
RIFLE$_{first24h}$-Injury	11
RIFLE$_{first24h}$-Failure	9
No AKI$_{first5days}$	15
RIFLE$_{first5days}$-Risk	8
RIFLE$_{first5days}$-Injury	14
RIFLE$_{first5days}$-Failure	7
1-year Mortality(%)	27
No AKI$_{first24h}$	34
RIFLE$_{first24h}$-Risk	21
RIFLE$_{first24h}$-Injury	27
RIFLE$_{first24h}$-Failure	18
No AKI$_{first5days}$	29
RIFLE$_{first5days}$-Risk	22
RIFLE$_{first5days}$-Injury	31
RIFLE$_{first5days}$-Failure	23
2-years Mortality(%)	35
No AKI$_{first24h}$	44
RIFLE$_{first24h}$-Risk	32
RIFLE$_{first24h}$-Injury	36
RIFLE$_{first24h}$-Failure	18
No AKI$_{first5days}$	38
RIFLE$_{first5days}$-Risk	36
RIFLE$_{first5days}$-Injury	41
RIFLE$_{first5days}$-Failure	23

Several recent studies seem to confirm that AKI is only associated with mortality when it is more severe. In a recent study of Abosaif et al[31], including critically ill patients, only RIFLE-F was associated with mortality. The presence of multiple organ failure seems to be the most important determinant of outcome in AKI patients, with increasing mortality as the number of failing organs increases.[25;32] In studies that did focus on sepsis patients, mostly only ICU, hospital or 90 day survival was considered and AKI definitions varied widely. The

combination of AKI and sepsis is reported to carry a mortality of 70% whereas the mortality of ARF alone is 40-45%.[14]

Daher et al found that the RIFLE criterion was not an independent predictor of mortality in patients admitted to an infectious disease ICU.[33] In a study by Kim et al, RIFLE class on the day of admission was not associated with an increased risk of 28 day mortality in patients with sepsis and septic shock.[34] Bagshaw et al used the ANZINCS (Australian New Zealand Intensive Care Society Adult Patient Database) for all adult admissions and found a low predictive value of RIFLE for in hospital death (adjusted odds ratio 1.54 (1.45-1.64)).[3]

Data on long term outcome in septic AKI/critically ill patients are scarce. In our cohort of exclusively sepsis patients we found a 1 year and 2 year mortality rate of 44% and 50% respectively, in the entire cohort (Table 3). In ICU survivors these were 27% and 35% respectively. There was no increasing trend in mortality from no-AKI over RIFLE-R, RIFLE-I and RIFLE-F (Table 3).

Bagshaw et al[35] found a 1 year mortality rate of 24.6% in 5693 retrospectively included critically ill patients. In patients with septic AKI, 1-year mortality rate was 49.7%. In a prospective study including 243 hospital surviving sepsis patients, Lopes et al found that 13.7% of patients died after a follow-up period of 21 $\pm$ 6.4 months.[36]

7.1.3.2 RRT and mortality

In our cohort, patients treated with RRT had a 1-year and 2-years mortality of 63% and 67% respectively, comparable to the study of Bagshaw et al who found a mortality rate of 64% at 1 year in those with severe ARF, defined as RRT need.[37] Overall, both short and long term outcome after AKI in patients treated with RRT in the ICU is poor with mortality rates varying between 40% and 85%.[38-53]

Mortality in patients treated with RRT is higher than in patients not treated with RRT.[4;54-57] AKI requiring RRT approximately affects around 6% of critically ill patients and results in a hospital mortality rate of 60%.[58] In a study by Neveu et al, mortality rate in septic ARF treated with RRT compared to non septic ARF treated with RRT was 82.4% vs 48.4%.[56] In this study, patients were generally more severily ill than in our cohort, as suggested by a higher APACHE II score, a longer LOS and a very high hospital mortality rate in the overall cohort (74.5%). In the SOAP study, mortality rate was comparable to our study with a rate of 61.9% in ARF due to all causes treated with RRT.[13] In a study by Clermont et al, critically ill patients were treated with RRT in 11% of cases which was associated with a hospital mortality rate of 57%.[55] Metcalfe et al found a 90 days mortality rate of 83% in all ARF patients treated with RRT, but mortality was substantially lower (55%) in those who were started in a renal unit compared to the ICU.[48] In a prospective study including 17126 critically ill patients treated with RRT, hospital mortality was 62.8%.[59]

7.2 Different AKI definitions and their impact on incidence and outcome

7.2.1 Impact of urinary output

Omitting the urinary output criterion in the the AKI definition decreases the prevalence of AKI and partly explains why epidemiological data on AKI vary widely between different studies and even within the same setting. In our cohort, the prevalence of AKI decreased when the urinary criterion was omitted, whichever definition was used (Table 4).

Although both RIFLE[60], AKIN[61] and also KDIGO[62] include the urinary output criterion in the definition for AKI and ERBP again stresses the importance of including the urinary output criterion[63], the latter has been omitted or modified in several studies on AKI epidemiology.[29;64;65] There are several explanations for the fact the urinary output criterion is often omitted. First, in large databases often only 24 hours urinary output is available which drives authors to use modified urinary output criteria.[64;65] Second, retrospective studies and administrative databases often lack data on urinary output. Third, measuring hourly urinary output is time-consuming and requires bladder catheterization which implies that hourly urinary output measurement is mostly only available in ICU setting and not in the general ward. However, Macedo et al[66;67] demonstrated that fixed 6 hour blocks for urinary output collection, corresponding to nurses' shifts, are equivalent to hourly measurements and thus can be easily applied both in the ICU and the general ward and in patients either with or without bladder catheter.

Urinary output can be considered as an online biomarker for AKI. As stated before, sCr is an imperfect parameter that only starts to increase hours after the initial insult.[68] We have demonstrated that urinary output is an early parameter that can diagnose AKI in a stage where there is no tubular damage yet.[69] Prowle et al studied 239 critically ill patients in 7 ICU's over 6 countries and found that oliguria of greater than 1h was associated with AKI defined as RIFLE≥I according to sCr the next day.[70] Leedahl et al found that oliguria of ≥ 3h in the first 12h after ICU admission predicted AKI as defined by KIDGO≥stage II based on sCr in patients with severe sepsis and septic shock.[71] Oliguria of greater than 5h had the best performance. APACHE III and sCr were not predictive in the first 12 hours.[71] In the study of Prowle, the majority of consecutive oliguria episodes was not followed by an AKI diagnosis based on sCr later on.[70] Although one possible explanation for this finding would be that olguria is less specific and can be false positive, another explanation can be that oliguria is a sensitive marker that detects AKI in an early stage and that early goal directed therapy prevented further evolution to AKI based on sCr.

Some studies found that the urinary output criterion is less predictive of mortality than the sCr criterion.[16;29;65;72-75] However, Macedo et al showed that urinary output is a sensitive and early marker of AKI and is associated with adverse outcomes in critically ill patients.[67] Oliguria of more than 12 hours and oliguria of 3 and more episodes were associated with an increased mortality rate. Cruz et al found that urinary output alone had a lower predictive

ability but that both criteria (thus including the urinary output criterion) had the best predictive ability.[72]

Several factors might influence the relationship between GFR and urinary output. First, although septic AKI is predominantly oliguric[14], AKI can also be non-oliguric.[76] In non-oliguric AKI, urinary output remains normal or even increases despite a decrease in GFR. Second, the use of diuretics might theoretically disturb the relationship between urinary output and GFR. However, Han et al[74] demonstrated that urinary output had an additional role in AKI staging irrespective of diuretic use. Third, fluid resuscitation can also affect urinary output independent of GFR. Pickering et al[77] developed a model combining creatinine and volume kinetics and studied 49 patients who were resuscitated after cardiac arrest. They showed that fluid resuscitation leads to an underestimation of sCr and AKI severity which thus leads to a delay in AKI diagnosis based on the sCr criterion. However, 41 of 44 patients classified as AKI by the sCr criterion, were oliguric in the first 48h, again underlining the importance of the urinary output criterion in the diagnosis of AKI, especially when the sCr can be lowered due to fluid dilution.[77]

Altogether, these findings stress the importance of urinary output in the diagnosis of AKI. Emphasis should be put on the application of both criteria (sCr *and* urinary output) for defining AKI.

7.2.2 Absolute vs relative increases in sCr and the impact of different baseline values

In our cohort, the prevalence of AKI ranged from 14% to 79% according to which AKI definition was used (Table 4). We changed AKI definitions either by 1) including vs not including the urinary output criterion, 2) using absolute vs relative increases in sCr, 3) using different baseline values (either a historical baseline, the ICU admission value or an estimated value) and 4) by changing the time span for AKI diagnosis; and we evaluated the impact on prevalence and outcome (Table 4).

Table 4: Influence of changing the AKI definition on prevalence of AKI, RRT need, ICU and 1 year mortality.

AKI definition	no AKI(n/%)	AKI(n/%)	p value
1.RIFLE_24h_sCr	110(56.4)	85(43.6)	
RRT need	8(7.3)	19(22.4)	0.002
ICU M	24(21.8)	21(24.7)	0.64
1Y M	48(43.6)	37(43.5)	0.99
2.RIFLE_24h_UO	110(56.4)	85(43.6)	
RRT need	10(9.1)	17(20)	0.03
ICU M	24(21.8)	21(24.7)	0.64
1Y M	48(43.6)	37(43.5)	0.99

3.RIFLE_24h_sCr_UO	64(32.8)	131(67.2)	
RRT need	3(4.7)	24(18.3)	0.01
ICU M	14(21.9)	31(23.7)	0.78
1Y M	31(48.4)	54(41.2)	0.34
4.RIFLE_5days_sCr	96(49.2)	99(50.8)	
noRRT need	3(3.1)	24(24.1)	<0.001
ICU M	18(18.8)	27(27.3)	0.16
1Y M	38(39.6)	47(47.5)	0.27
5.RIFLE_5days_UO	73(37.4)	122(62.6)	
RRT need	3(4.1)	24(19.7)	0.002
ICU M	16(21.9)	29(23.8)	0.77
1Y M	31(42.5)	54(44.3)	0.81
6.RIFLE_5days_sCr_UO	42(21.5)	153(78.5)	
RRT need	1(2.4)	26(17)	0.02
ICU M	8(19)	37(24.2)	0.48
1Y M	18(42.9)	67(43.8)	0.91
1Y M			
7.AKIN_48h_sCr	154(79)	41(21)	
RRT need	9(5.8)	18(43.9)	<0.001
ICU M	30(19.5)	15(36.6)	0.02
1Y M	63(40.9)	22(53.7)	0.14
8.AKIN_48h_UO	94(48.2)	101(51.8)	
RRT need	7(7.4)	20(19.8)	0.01
ICU M	22(23.4)	23(22.8)	0.92
1Y M	39(41.5)	46(45.5)	0.57
9.AKIN_48h_sCr_UO	85(43.6)	110(56.4)	
RRT need	6(7.1)	21(19.1)	0.02
ICU M	18(21.2)	27(24.5)	0.58
1Y M	33(38.8)	52(47.3)	0.24
10.KDIGO_sCr	90(46.2)	105(53.8)	
RRT need	3(3.3)	24(23.1)	<0.001
ICU M	17(18.9)	28(26.2)	0.20
1Y M	37(41.1)	48(45.7)	0.52
11.KDIGO_UO	73(37.4)	122(62.6)	
RRT need	3(4.1)	24(19.7)	0.002
ICU M	16(21.9)	29(23.8)	0.77
1Y M	31(42.5)	54(44.3)	0.81
12.KDIGO_sCr_UO	42(21.5)	153(78.5)	
RRT need	1(2.4)	26(17)	0.02
ICU M	8(19)	37(24.2)	0.48
1Y M	18(42.9)	67(43.8)	0.91
13.ERBP_5days_sCr	141(72.3)	54(27.7)	

RRT need	7(5)	20(37)	<0.001
ICU M	23(16.3)	22(40.7)	<0.001
1Y M	55(39)	30(55.6)	0.04
14.ERBP_5days_UO	73(37.4)	122(62.6)	
RRT need	3(4.1)	24(19.7)	0.002
ICU M	16(21.9)	29(23.8)	0.77
1Y M	31(42.5)	54(44.3)	0.81
15.ERBP_5days_sCr_UO	63(32.3)	132(67.7)	
RRT need	3(4.8)	24(18.2)	0.01
ICU M	11(17.5)	34(25.8)	0.20
1Y M	25(39.7)	60(45.5)	0.45
16.ΔHIS_24h_sCr	97(49.7)	98(50.3)	
RRT need	4(4.1)	23(23.5)	<0.001
ICU M	20(20.6)	25(25.5)	0.42
1Y M	43(44.3)	42(42.9)	0.84
17.ΔHIS_24h_sCr_UO	59(29.2)	138(70.8)	
RRT need	1(3.7)	26(18.8)	0.002
ICU M	13(22.8)	32(32.2)	0.95
1Y M	29(50.9)	56(40.6)	0.19
18.ΔHIS_48h_sCr	91(46.7)	104(53.3)	
RRT need	3(3.3)	24(23.1)	<0.001
ICU M	18(19.8)	27(26)	0.31
1Y M	39(42.9)	46(44.2)	0.85
19.ΔHIS_48h_sCr_UO	47(24.1)	148(75.9)	
RRT need	1(2.1)	26(17.6)	0.008
ICU M	11(23.4)	34(23)	0.45
1Y M	22(46.8)	63(42.6)	0.61
20.ΔEST_24h_sCr	106(54.4)	89(45.6)	
RRT need	3(2.8)	24(27)	<0.001
ICU M	23(21.7)	22(24.7)	0.62
1Y M	44(41.5)	41(46.1)	0.52
21.ΔEST_24h_sCr_UO	65(33.3)	130(67.7)	
RRT need	1(1.5)	26(20)	<0.001
ICU M	16(24.6)	29(22.3)	0.72
1Y M	30(46.2)	55(42.3)	0.61
22.ΔEST_48h_sCr	103(52.8)	92(47.2)	
RRT need	2(1.9)	25(27.2)	<0.001
ICU M	21(20.4)	24(26.1)	0.35
1Y M	41(39.8)	44(47.8)	0.26
23.ΔEST_48h_sCr_UO	56(28.7)	139(71.3)	
RRT need	1(1.8)	26(18.7)	0.002
ICU M	14(25)	31(22.3)	0.69
1Y M	23(41.1)	62(44.6)	0.65

24.ΔADM_day1_sCr	166(85.1)	27(13.8)	
RRT need	13(7.8)	14(51.9)	<0.001
ICU M	31(18.7)	12(44.4)	0.003
1Y M	66(39.8)	17(63)	0.02
25.ΔADM_day1_sCr_UO	105(53.8)	88(45.1)	
RRT need	9(8.6)	18(20.5)	0.02
ICU M	20(19)	23(26.1)	0.24
1Y M	42(40)	41(45.6)	0.36

(Legend:UO: urinary output criterion, sCr: serum creatinine criterion, 24h: AKI diagnosis over a time span of 24 hours after ICU admission, 48h: AKI diagnosis over a time span of 48h after ICU admission, 5days: AKI diagnosis over a time span of 5 days after ICU admission, ΔEST: creatinine increase based on the difference between the highest value over a certain time span and an estimated baseline value according to ADQI, ΔADM_day 1: creatinine increase based on the difference between the value 24 hours after admission and the ICU admission value, ΔHIS: creatinine increase based on the difference between the highest value over a certain time span and a historical baseline value.)

As described in Chapter 7.1.3.2 on mortality and AKI, we did not find an increase in mortality with increasing stages of AKI according to RIFLE (no AKI vs RIFLE-R, RIFLE-I and RIFLE-F). Whether AKI is an independent predictor of mortality remains controversial and when an association is found, it is mostly for more severe AKI and short term mortality. Poukkanen et al found that in a cohort of 918 sepsis patients, only KDIGO stage 3 was associated with 90 day mortality, whereas stage 1 and 2 were not.[78] In a study by Hoste et al[4], the excess mortality in patients with AKI could be entirely explained by the high mortality in patients treated with RRT. Increasing age, need for vasoactive treatment and need for RRT were independently associated with mortality in their model.[4] Ostermann et al demonstrated that, although there is an association between AKI and hospital outcome, associated organ failure, non-surgical admission and admission after emergency surgery, all had a greater impact on prognosis than severity of AKI.[7] In another study, it was demonstrated that the most important predictors for mortality were already present at admission to the ICU and included advanced age, the presence of infection, a past history of certain chronic diseases and the presence of other failing organs.[32]

Although in recent literature it was demonstrated that even small increases in serum creatinine are independently associated with a higher risk of mortality, these results are mainly obtained in cohorts of cardiac surgery patients, after coronarography and after myocardial infarction [21;79-82] and cannot necessarily be extrapolated to sepsis. In our cohort we analysed different cut-off levels for *absolute* sCr increase (0.1 to 0.5 mg/dl with an interval of 0.1mg/dl) for association with mortality and we used different reference values to calculate this serum creatinine increase (either a historical baseline value, an estimated baseline value according to ADQI or the ICU admission value).

We found that, as in the cardiac surgery setting, a 0.3 mg/dl increase in sCr was associated with mortality but only if this increase was calculated based on the difference between the value 24 hours after admission and the ICU admission value. Compared to all of the other

definitions we used, this is the only definition that incorporates the response to therapy (e.g. fluid resuscitation or vasopressor therapy) by comparing the sCr 24h after admission with the ICU admission value. These findings suggest that the evolution of sCr in the first 24h is more predictive of outcome than the highest value over a certain time span. Most likely, the evolution of the sCr in the first 24h is a reflection of the fluid responsiveness, as explained by the KDIGO guideline on AKI and confirmed by the ERBP position statement on this topic.[62;63]

Although we did find an association between a 0.3mg/dl increase in sCr and mortality, this association did not persist after adjustment for severity of illness. This finding was confirmed by assessing AUC ROC curves demonstrating no change in discriminatory power by adding vs not adding AKI to the model (AUC ROC 0.71 for both).

In our cohort, 150 patients survived ICU. In a multivariate analysis, including different AKI definitions to the model, only age but not AKI, was retained as an independent predictor of mortality. These results are corroborated by Sasse et al who demonstrated that the survival rate was negatively correlated with the APACHE II score up to 1 month after hospital admission, but uncorrelated thereafter.[83] Ponte et al also showed that long-term outcome seems to be more conditioned by general factors rather than by the acute illness.[84] Pereira et al found that long term survival of AKI patients was associated with their underlying comorbidities and not with severity of AKI.[85] In the same line, health related quality of life after recovery is more strongly affected by pre-existing diseases and age than by severity of illness in ICU.[38] Although Bagshaw et al found that 1 year mortality was independently associated with advanced age, medical diagnosis, higher APACHE II score and presence and severity of kidney function, there was no difference in outcome between those with mild versus moderate kidney dysfunction defined as a peak sCr between 1.7mg/dl and 3.4 mg/dl versus a peak sCr of >3.4mg/dl but without RRT need, respectively.[35] Thus although patients with either mild or moderate kidney dysfunction had an increased risk of death vs those without kidney dysfunction, use of sCr level alone was poor at discriminating long term outcome, suggesting this measure alone should not be used for defining long-term prognosis.[35] However, our results were not corroborated by Linder et al, who did find an association between small increases of sCr and long-term mortality, even after adjustment for several covariates.[86]

7.3 Biomarkers

7.3.1 Introduction

The search for new biomarkers has been driven by the fact that AKI diagnosis currently relies on imperfect parameters such as sCr which is a functional parameter that only rises hours after the initial insult.[68] Also, the percentage changes in sCr after severe AKI are highly dependent on baseline kidney function. In a study of Waikar et al[87], twenty-four hours after a 90% reduction in creatinine clearance, the rise in sCr was 246% with normal baseline

kidney function, 174% in stage 2 CKD, 92% in stage 3 CKD and only 47% in stage 4 CKD as opposed to a nearly identical absolute increase in sCr (1.8 to 2 mg/dl).
The paradigm that sCr is an imperfect parameter has stimulated the search for a troponin-like biomarker for AKI diagnosis. An ideal biomarker for AKI should 1) only increase in case of tubular damage, 2) correspond to the potential lesions seen on biopsy, 3) follow the clinical course, 4) not increase in case of other organ failure besides renal and 5) be easily measured at low cost.[88]
In the last decade, many papers on the use of new urinary and serum biomarkers for AKI were published, mostly concluding that these biomarkers will lead to a new era with earlier diagnosis, better prognostication of outcome in terms of need for renal replacement and/or mortality, and finally better survival.[89] Nevertheless, there remains a gap between the fascinating findings at the basic science level and the clinical application of this knowledge and objective evaluation of the available literature shows a rather disappointing picture.[90]
There are different potential explanations for these disappointing results. First, biomarkers are studied in widely different clinical settings, from paediatric post cardiac surgery, where timing and amount of renal impact are exactly known, to patients with septic shock, where timing of renal insult is unknown. Overall, diagnostic performance of biomarkers appears to be better in situations with a known timing and etiology of renal injury. Second, most of these markers are not only associated with kidney damage, but also with the underlying conditions causing the AKI, such as sepsis, diabetic nephropathy, systemic lupus erythematosus and hemolytic uremic syndrome.[91-94] Some of these markers are also increased in chronic kidney disease[95;96], blurring the differential diagnosis between CKD and AKI. The reference baseline creatinine of a given patient is not always available, so in these patients, it is unclear whether an increased creatinine is due to AKI or chronic kidney disease (CKD). The behavior of most biomarkers in patients with CKD is however largely unknown. Usefulness of biomarkers should thus be addressed differently for different clinical settings, as it is apparent that performance is strongly dependent on the underlying circumstances. As such, results in one setting cannot be generalised. Especially in sepsis, the use of biomarkers is hampered by the fact that there is no clear timing of insult and that patients are very heterogenous with different comorbidities. AKI is mostly multifactorial, especially in the critically ill setting, and thus it is not likely that one single biomarker will cover its entire spectrum.[97] Some authors have tried to resolve this issue by using a panel of biomarkers instead of one single biomarker.[98] Although the results seem promising, there remains an important overlap between AKI and no AKI patients. Also, the high cost associated with these techniques is clearly a limiting factor and in the absence of a clear benefit over more traditional markers, their use cannot be justified. Kashani et al[99] recently found that prediction of AKI in critically ill patients was improved by combining two urinary biomarkers, involved in the G1 cell cycle arrest. The two-marker panel performed better than either one of the markers separately and also performed better than urinary or plasma NGAL, plasma cystatin C and urinary KIM-1, pi-GST, LFABP and IL-18. In the latter study, AKI was defined as KDIGO stage 2 or 3 whereas the focus should e on diagnosing early stages of AKI at a time when serum creatinine is not yet increased.[99] However, it must also be acknowledged that

as long as we lack a clinical useful gold standard for diagnosing AKI, it remains difficult to prove the advantage of any new biomarker over the imperfect parameter that is used (sCr).

7.3.2 Neutrophil gelatinase-associated Lipocalin (NGAL)

One of the most studied biomarkers in the field of nephrology is Neutrophil gelatinase-associated Lipocalin (NGAL).[100;101] NGAL was first discovered in the granules of neutrophils and was originally used for differentiation between bacterial and viral infections.[102-104] However, it can also be induced by distal renal tubular cells in case of kidney injury and is expressed by several other epithelia such as the uterus, prostate, salivary glands, lung, liver, trachea, stomach and colon.[105] Also, in urinary tract infection the presence of urinary leucocytes influences urinary NGAL levels. [106]

Several immunoassays have been developed for the measurement of NGAL. Currently, there are three commercially available immunoassays: 1) Triage NGAL test from Biosite-Inverness Medical which is a point of care fluorescence immunoassay that can be used bedside (result after approximately 20 minutes) on whole blood or plasma, 2) NGAL Test from Bioporto, an enhanced turbidimetric immunoassay with the advantage of potential use on different clinical chemistry analyzers and suitable for testing both plasma and urine with only a small amount of sample (3µl) requested (result in 10 minutes), 3) ARCHITECT platform from Abbott Diagnostics, a chemiluminescent microparticle enhanced immunoturbidimetric immunoassay wich requires a sample volume of 150µl and only operates on urine (result in 35 minutes). Besides these commercially available automated immune assays there are also several non-automated research ELISA's.[107-111]

In humans, NGAL can be found in three different forms: 1) a 25kDa monomer, 2) a 45 kDa disulfide-linked homodimer and 3) a 135 kDa heterodimer where the protein is covalently bonded to matrix metalloproteinase 9 (MMP-9). The fact that different forms of NGAL exist, implies that the antibody configuration of an immunoassay might affect the clinical performance, depending on which form of NGAL is potentially measured. Cai et al [112] showed that the monomeric form and to some extent the heterodimeric forms are the predominant forms produced by tubular epithelial cells, whereas the dimeric form seems unique to the neutrophils. In their study, urine levels of NGAL were measured comparing five ELISA's each using different antibodies which reacted with different epitopes and thus identifying different molecular structures. Martensson et al [113] tried to 'remove' the dimeric signal in urine by constructing a ratio of two different ELISA tests, which amplified the monomeric signal and almost completely distinguished monomeric from dimeric NGAL. However, the currently available immunoassays do not distinguish between the protein produced by the renal tubular cell versus the protein synthesized by neutrophils and even if such a test would be commercially available, some other issues remain problematic in the use of NGAL as a marker of AKI. First, neutrophils (and thus dimeric NGAL) are increasingly being recognized as having an important role in the pathophysiology of AKI.[114] This implies that potentially also dimeric NGAL can indirectly be a reflection of tubular damage. Second,

most patients with CKD demonstrate a common pathway of chronic tubulointersitial damage and thus increased (supposingly) monomeric NGAL levels even in the absence of acute (on chronic) kidney disease. Third, there are insufficient data about biological variability, additional pre analytical sources of variation and definitive diagnostic tresholds are unavailable. [115]

In sepsis, several studies demonstrated increased levels of NGAL , even in the absence of AKI.[116;117] Since NGAL is filtered at the glomerular level, its presence in the urine can also be due to overflow from the systemic circulation, even in the absence of AKI. In our study, we found an important correlation between uNGAL and sNGAL. NGAL levels increased with increasing tertiles of CRP and APACHE II score increase, illustrating the relationship between NGAL and increasing levels of severity of inflammation and severity of illness. The latter pleads for caution when using NGAL as a diagnostic criterion for AKI in sepsis

7.3.3 Pathophysiology of AKI in sepsis

7.3.3.1 Transient and intrinsic AKI

One of the major challenges in clinical nephrology is the differentiation between transient and intrinsic AKI. Transient or so called 'prerenal azotemia' is considered a physiological response to a decrease in glomerular capillary perfusion pressure with little or no tubular damage, presumed that the condition is managed in a timely manner by increasing perfusion pressure, either by fluid resuscitation or vasopressor therapy.[118-122] We demonstrated that urinary NGAL levels were not different between no AKI and transient AKI if the diagnosis was based on the urinary output criterion, however when based on the sCr criterion, we found a gradual increase in uNGAL levels. This suggests that transient AKI without tubular damage does exist and at the same time, underlines the importance of urinary output as an online biomarker for detecting AKI in a stage where there is no tubular damage yet. The existence of transient AKI without tubular damage has been questioned in recent literature. Doi et al and Nejat et al[123;124] demonstrated that biomarker levels were increased, even in patients who were classified as having transient AKI, suggesting that there is always a certain degree of tubular damage, even in transient AKI, and that immunological cascades, causing direct tubular damage, rather than renal hypoperfusion, are responsible for septic AKI.[125] This belief has also led to the creation of a new entity called 'subclinical AKI' which is defined as a condition where the sCr and urinary output criteria of RIFLE, AKIN,KDIGO or ERBP are not fulfilled but biomarkers are positive.[126;127] As stated above, we found a strong correlation between serum and urinary NGAL in sepsis and concluded that biomarker positivity does not automatically imply tubular damage, at least in sepsis, and thus can be falsely positive. The belief that transient AKI without tubular damage does not exist has also been stimulated by the fact that several reports demonstrated that not only intrinsic[128] but also transient AKI portends a worse prognosis compared to no AKI.[79;129;130 131] However, it remains uncertain whether this reflects a causal relationship or whether transient AKI is predominantly a surrogate marker for severity of disease. In a retrospective study of Uchino et al[130],

hospitalized patients with transient azotemia, defined as a recovery of kidney function within 72h, had higher odds for hospital mortality. However, baseline serum creatinine values were not available in the majority of patients and were estimated according to ADQI[60] which might have influenced the classification. Also, data on comorbidities influencing the prevalence of AKI, such as diabetes, were not available.

7.3.3.2 Urinary indices

In our study, we calculated the FENa and FEUrea in 107 septic patients and classified them according to FEUrea and FENa quartiles. Our data indicate that in patients with incipient sepsis, FENa is far below the proposed cut-off of 1%. Less than 25% of patients had a FENa above 1%, whereas 50% had a FENa below 0.36%. This is in line with what we would expect in a situation of renal hypoperfusion with tubular integrity, as hypoperfusion at the level of the glomerulus will upregulate tubular retention of sodium, leading to a decrease in FENa. When tubular damage occurs, the polarization of the tubular cell is reversed, and sodium is no longer reabsorbed, but rather actively secreted into the tubular space. It seems likely that a very low FENa always is a hallmark of renal hypoperfusion with absence of tubular damage, whereas a higher FENa around 1% might be both a sign of improved renal perfusion with intact tubuli or of beginning loss of polarization, and thus tubular damage. Bagshaw et al[132] recently reported an admission FENa below 1% in 57% of septic patients, being not different from non-septic patients. Darmon et al[133] reported a median FENa on admission of 0.50%. Using RoC curve analysis, the same authors also found that a fractional excretion of 0.58% had the best discriminative power, resulting in a positive and negative predictive power for persistent AKI of 0.71 and 0.47 respectively.[133] In our cohort, using the median of 0.36%, we observed a dose dependent increase in prevalence of AKI based on the urinary outputcriterion, with quartiles of decreasing FENa and FEUrea. There was no association for AKI based on the sCr criterion. These findings illustrate that patients become more oliguric as they retain more salt and water. In this cohort, neither one of the used tools (either FENa, FEUrea or NGAL) could differentiate between transient and intrinsic AKI. However, a combined use of FENa and FEUrea had a predictive value.
Several tools, such as urinary indices and urinary microscopic evaluation have been studied to differentiate between transient and intrinsic AKI. Espinel et al introduced the use of FENa for differentiation between prerenal and intrinsic AKI in 1976.[134] In the prerenal state, the tubule retains salt and water whereas in intrinsic AKI polarity might be lost which in turn leads to active sodium secretion and hence a high FENa. However, differential diagnosis is hampered by conditions in which intrinsic AKI is associated with a low FENa (f.e. myoglobinuric renal failure, hemoglobinuric renal failure, contrast nephrotoxicity, non oliguric ATN, hepatorenal syndrome, heart failure, renal aretery stenosis) and prerenal azotemia with a high FENa (volume depletion with ongoing diuretic use, metabolic alkalosis, glucosuric states, aldosterone deficiency).[135-138] Vaz et al[139] included sepsis to the list of conditions affecting the reliability of the FENa test. Their conclusion was based on the results of a low FENa in only two septic patients. Authors excluded prerenal azotemia as a

possible explanation for their findings because adequate central filling pressures were found by Swann Ganz catheterisation. However, there is no reliable tool to estimate volume status and a low glomerular perfusion pressure in these patients, despite a general hyperdynamic state, cannot be excluded.

The use of FEUrea could theoretically overcome the problem of a high FENa in conditions of volume depletion with continued diuretic use, since tubular urea transport is mainly passive and thus less affected by the use of diuretics.[136-142] A direct relation between urinary flow rate and urea excretion is well known.[143] There are conflicting results on the use of FENa and FEUrea for dfferential diagnosis between prerenal/transient and intrinsic AKI. Carvounis et al[144] found that a low FEUrea had a higher sensitivity and specificity for differentiating between prerenal azotemia and intrinsic AKI, especially when diuretics were used, in 102 consecutive adult patients referred to the nephrologist for evaluation. They also demonstrated that FEUrea decreased proportionally to urinary output. However, Pepin et al[145] found that FEUrea is not a better tool than FENa to diagnose transient AKI, regardless of diuretic intake. In a systematic review, both in humans and in experimental septic AKI, Bagshaw et al[146;147] concluded that neither the use of urinary indices or urinary microscopic evaluation is of any clear benefit in the differential diagnosis between prerenal and intrinsic septic AKI. However, only 52% of patients in their review fulfilled the criteria for sepsis and only 54% had clear evidence of AKI.[146] Most of the included studies did not have a control group and the time frame for diagnosis of sepsis and ARF was highly variable, inconsistent or not reported.[148]

Surprisingly, and against our initial hypothesis, a low (<31.5 %) fractional urea excretion at 4 hours was associated with intrinsic rather than with transient AKI, especially when it was persistent over time, whereas a value higher than 31.5% had a very high negative predictive value for intrinsic AKI. In patients with a fractional sodium excretion above 0.36%, the discriminatory value of fractional urea excretion was even more pronounced (figure 4B).

Darmon et al reported a FEUrea of 39% in the no AKI vs 41% in the transient AKI vs 32% in the intrinsic AKI group, and concluded that FEUrea had a low value for discriminating intrinsic AKI.[133] They based their analysis on admission FEUrea, whereas we used the value 4 hours after admission, when some attempts to restore low glomerular filtration pressure (e.g. by a fluid challenge or start of vasopressors) have already been installed. However, data based on FEUrea and FENa at admission showed comparable results, though less impressive (data not shown). Our findings are in line with those of De Witte et al[149], who also found a reasonable value of FEurea<40% as a parameter to discriminate transient from intrinsic AKI. As a low fractional excretion of urea apparently represents a low glomerular filtration, we hypothesized that a combination of fractional sodium and urea excretion would yield the most optimal prediction of patients with a substantial chance to respond positively to attempts to restore glomerular filtration. Indeed, in our cohort, a combination of a high fractional sodium and urea excretion yielded a 95% probability of restoring diuresis, and AKI being transient rather than intrinsic, whereas a persistently low fractional urea excretion in combination with a high fractional sodium excretion was suggestive for intrinsic AKI. We also

demonstrated that there was a continuum between transient AKI without tubular damage, transient AKI with minor tubular damage and intrinsic AKI.

7.4 Limitations

We fully acknowledge that this work is based on a relatively small cohort of patients. Also, due to our inclusion criteria mainly patients with severe sepsis and septic shock were included which means that results can not be generalized to patients with less se vere sepsis. Although 195 patients were included, NGAL levels were only measured in the first 107 patients because interim analyses failed to show a benefit for NGAL in diagnosing AKI or predicting RRT. This implies that the studies described in chapters 4, 5 and 6 are focusing on the first 107 patients only. For the study on robustness of creatinine based AKI definitions (chapter 2), all 195 patients were considered. Further prospective studies in sepsis including a larger number of patients are warranted to validate our findings.

7.5 Reference List

1. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; 20: 864-874

2. Bagshaw SM, George C, Dinu I, Bellomo R. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2008; 23: 1203-1210

3. Bagshaw SM, George C, Bellomo R. Early acute kidney injury and sepsis: a multicentre evaluation. *Crit Care* 2008; 12: 1-9

4. Hoste EA, Lameire NH, Vanholder RC, Benoit DD, Decruyenaere JM, Colardyn FA. Acute renal failure in patients with sepsis in a surgical ICU: predictive factors, incidence, comorbidity, and outcome. *Journal of the American Society of Nephrology* 2003; 14: 1022-1030

5. Yegenaga I, Hoste E, Van BW *et al*. Clinical characteristics of patients developing ARF due to sepsis/systemic inflammatory response syndrome: results of a prospective study. *Am J Kidney Dis* 2004; 43: 817-824

6. Lopes JA, Jorge S, Resina C *et al*. Acute kidney injury in patients with sepsis: a contemporary analysis. *Int J Infect Dis* 2009; 13: 176-181

7. Ostermann M, Chang RW. Acute kidney injury in the intensive care unit according to RIFLE. *Crit Care Med* 2007; 35: 1837-1843

8. Lopes JA, Jorge S, Resina C *et al*. Acute kidney injury in patients with sepsis: a contemporary analysis. *International Journal of Infectious Diseases 2009;13(2):176-81*

9. Oppert M, Engel C, Brunkhorst FM *et al*. Acute renal failure in patients with severe sepsis and septic shock--a significant independent risk factor for mortality: results from the German Prevalence Study. *Nephrology Dialysis Transplantation* 2008; 23: 904-909

10. Hsu CY, Ordonez JD, Chertow GM, Fan D, McCulloch CE, Go AS. The risk of acute renal failure in patients with chronic kidney disease. *Kidney Int* 2008; 74: 101-107

11. Khosla N, Soroko SB, Chertow GM *et al*. Preexisting chronic kidney disease: a potential for improved outcomes from acute kidney injury. *Clinical Journal of The American Society of Nephrology: CJASN* 2009; 4: 1914-1919

12. Singh P, Rifkin DE, Blantz RC. Chronic kidney disease: an inherent risk factor for acute kidney injury? *Clin J Am Soc Nephrol* 2010; 5: 1690-1695

13. Vincent JL, Sakr Y, Sprung CL *et al*. Sepsis in European intensive care units: results of the SOAP study. *Crit Care Med* 2006; 34: 344-353

14. Bagshaw SM, Uchino S, Bellomo R *et al*. Septic acute kidney injury in critically ill patients: clinical characteristics and outcomes. *Clinical Journal of The American Society of Nephrology: CJASN* 2007; 2: 431-439

15. Mehta RL, Pascual MT, Gruta CG, Zhuang S, Chertow GM. Refining predictive models in critically ill patients with acute renal failure. *J Am Soc Nephrol* 2002; 13: 1350-1357

16. Hoste EA, Clermont G, Kersten A *et al*. RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients: a cohort analysis. *Critical Care* 2006; 10: 1-10

17. Brun-Buisson C, Meshaka P, Pinton P, Vallet B, EPISEPSIS Study Group. EPISEPSIS: a reappraisal of the epidemiology and outcome of severe sepsis in French intensive care units. *Intensive Care Medicine* 2004; 30: 580-588

18. Friedman G, Silva E, Vincent JL. Has the mortality of septic shock changed with time. *Crit Care Med* 1998; 26: 2078-2086

19. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; 348: 1546-1554

20. Gaieski DF, Edwards JM, Kallan MJ, Carr BG. Benchmarking the incidence and mortality of severe sepsis in the United States. *Crit Care Med* 2013; 41: 1167-1174

21. Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol* 2005; 16: 3365-3370

22. Clec'h C, Darmon M, Lautrette A *et al*. Efficacy of renal replacement therapy in critically ill patients: a propensity analysis. *Crit Care* 2012; 16: 1-9

23. Hou SH, Bushinsky DA, Wish JB, Cohen JJ, Harrington JT. Hospital-acquired renal insufficiency: a prospective study. *American Journal of Medicine* 1983; 74: 243-248

24. Kellum JA, Angus DC. Patients are dying of acute renal failure. *Crit Care Med* 2002; 30: 2156-2157

25. Levy EM, Viscoli CM, Horwitz RI. The effect of acute renal failure on mortality. A cohort analysis. *JAMA* 1996; 275: 1489-1494

26. Ali T, Khan I, Simpson W *et al*. Incidence and outcomes in acute kidney injury: a comprehensive population-based study. *J Am Soc Nephrol* 2007; 18: 1292-1298

27. Wald R, Quinn RR, Luo J *et al*. Chronic dialysis and death among survivors of acute kidney injury requiring dialysis. *JAMA* 2009; 302: 1179-1185

28. Lo LJ, Go AS, Chertow GM *et al*. Dialysis-requiring acute renal failure increases the risk of progressive chronic kidney disease. *Kidney Int* 2009; 76: 893-899

29. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. *Kidney Int* 2008; 73: 538-546

30. Lopes JA, Jorge S, Resina C *et al*. Prognostic utility of RIFLE for acute renal failure in patients with sepsis. *Crit Care* 2007; 11: 408: 1

31. Abosaif NY, Tolba YA, Heap M, Russell J, El Nahas AM. The outcome of acute renal failure in the intensive care unit according to RIFLE: model application, sensitivity, and predictability. *Am J Kidney Dis* 2005; 46: 1038-1048

32. De Mendonca A, Vincent JL, Suter PM *et al*. Acute renal failure in the ICU: risk factors and outcome evaluated by the SOFA score. *Intensive Care Med* 2000; 26: 915-921

33. Daher EF, Marques CN, Lima RS *et al*. Acute kidney injury in an infectious disease intensive care unit - an assessment of prognostic factors. *Swiss Medical Weekly* 2008; 138: 128-133

34. Kim WY, Huh JW, Lim CM, Koh Y, Hong SB. Analysis of progression in risk, injury, failure, loss, and end-stage renal disease classification on outcome in patients with severe sepsis and septic shock. *Journal of Critical Care* 2012; 27: 104-107

35. Bagshaw SM, Mortis G, Doig CJ, Godinez-Luna T, Fick GH, Laupland KB. One-year mortality in critically ill patients by severity of kidney dysfunction: a population-based assessment. *American Journal of Kidney Diseases* 2006; 48: 402-409

36. Lopes JA, Fernandes P, Jorge S *et al*. Long-term risk of mortality after acute kidney injury in patients with sepsis: a contemporary analysis. *BMC Nephrology* 2010; 11: 9

37. Bagshaw SM, Laupland KB, Doig CJ *et al*. Prognosis for long-term survival and renal recovery in critically ill patients with severe acute renal failure: a population-based study. *Crit Care* 2005; 9: R700-R709

38. Ahlstrom A, Tallgren M, Peltonen S, Rasanen P, Pettila V. Survival and quality of life of patients requiring acute renal replacement therapy. *Intensive Care Med* 2005; 31: 1222-1228

39. Bell M, Liljestam E, Granath F, Fryckstedt J, Ekbom A, Martling CR. Optimal follow-up time after continuous renal replacement therapy in actual renal failure patients stratified with the RIFLE criteria. *Nephrol Dial Transplant* 2005; 20: 354-360

40. Bellomo R, Cass A, Cole L *et al*. Intensity of continuous renal-replacement therapy in critically ill patients. *N Engl J Med* 2009; 361: 1627-1638

41. Benoit DD, Hoste EA, Depuydt PO *et al*. Outcome in critically ill medical patients treated with renal replacement therapy for acute renal failure: comparison between patients with and those without haematological malignancies. *Nephrol Dial Transplant* 2005; 20: 552-558

42. Carl DE, Grossman C, Behnke M, Sessler CN, Gehr TW. Effect of timing of dialysis on mortality in critically ill, septic patients with acute renal failure. *Hemodial Int* 2010; 14: 11-17

43. Kaltenmaier B, Pommer W, Kaufmann F, Hennig E, Molzahn M, Hetzer R. Outcome of patients with ventricular assist devices and acute renal failure requiring renal replacement therapy. *ASAIO J* 2000; 46: 330-333

44. Korkeila M, Ruokonen E, Takala J. Costs of care, long-term prognosis and quality of life in patients requiring renal replacement therapy during intensive care. *Intensive Care Medicine* 2000; 26: 1824-1831

45. Landoni G, Zangrillo A, Franco A *et al*. Long-term outcome of patients who require renal replacement therapy after cardiac surgery. *Eur J Anaesthesiol* 2006; 23: 17-22

46. Lin YF, Ko WJ, Chu TS *et al*. The 90-day mortality and the subsequent renal recovery in critically ill surgical patients requiring acute renal replacement therapy. *Am J Surg* 2009; 198: 325-332

47. Luckraz H, Gravenor MB, George R *et al*. Long and short-term outcomes in patients requiring continuous renal replacement therapy post cardiopulmonary bypass. *Eur J Cardiothorac Surg* 2005; 27: 906-909

48. Metcalfe W, Simpson M, Khan IH *et al*. Acute renal failure requiring renal replacement therapy: incidence and outcome. *Qjm* 2002; 95: 579-583

49. Morgera S, Kraft AK, Siebert G, Luft FC, Neumayer HH. Long-term outcomes in acute renal failure patients treated with continuous renal replacement therapies. *American Journal of Kidney Diseases* 2002; 40: 275-279

50. Saudan P, Niederberger M, De SS *et al*. Adding a dialysis dose to continuous hemofiltration increases survival in patients with acute renal failure. *Kidney Int* 2006; 70: 1312-1317

51. Schiffl H, Fischer R. Five-year outcomes of severe acute kidney injury requiring renal replacement therapy. *Nephrol Dial Transplant* 2008; 23: 2235-2241

52. Triverio PA, Martin PY, Romand J, Pugin J, Perneger T, Saudan P. Long-term prognosis after acute kidney injury requiring renal replacement therapy. *Nephrol Dial Transplant* 2009; 24: 2186-2189

53. Wong LP, Blackley MP, Andreoni KA, Chin H, Falk RJ, Klemmer PJ. Survival of liver transplant candidates with acute renal failure receiving renal replacement therapy. *Kidney Int* 2005; 68: 362-370

54. Bivet FG. Nonuse of RIFLE classification urine output criteria: biases for acute kidney injury biomarkers performance assessment? *Crit Care Med* 2012; 40: 1692-1693

55. Clermont G, Acker CG, Angus DC, Sirio CA, Pinsky MR, Johnson JP. Renal failure in the ICU: comparison of the impact of acute renal failure and end-stage renal disease on ICU outcomes. *Kidney International* 2002; 62: 986-996

56. Neveu H, Kleinknecht D, Brivet F, Loirat P, Landais P. Prognostic factors in acute renal failure due to sepsis. Results of a prospective multicentre study. The French Study Group on Acute Renal Failure. *Nephrology Dialysis Transplantation* 1996; 11: 293-299

57. Groeneveld AB. Pathogenesis of acute renal failure during sepsis. *Nephrol Dial Transplant* 1994; 9 Suppl 4: 47-51

58. Uchino S, Kellum JA, Bellomo R *et al*. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA* 2005; 294: 813-818

59. Metnitz PG, Krenn CG, Steltzer H *et al*. Effect of acute renal failure requiring renal replacement therapy on outcome in critically ill patients. *Crit Care Med* 2002; 30: 2051-2058

60. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; 8: R204-R212

61. Mehta RL, Kellum JA, Shah SV *et al*. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 2007; 11: 1-8

62. Kidney disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Int Suppl* 2012; 2: 1-138

63. Fliser D, Laville M, Covic A *et al*. A European Renal Best Practice (ERBP) position statement on the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines on acute kidney injury: part 1: definitions, conservative management and contrast-induced nephropathy. *Nephrol Dial Transplant* 2012; 27: 4263-4272

64. Bagshaw SM, George C, Bellomo R. A comparison of the RIFLE and AKIN criteria for acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2008; 23: 1569-1574

65. Joannidis M, Metnitz B, Bauer P *et al*. Acute kidney injury in critically ill patients classified by AKIN versus RIFLE using the SAPS 3 database. *Intensive Care Med* 2009; 35: 1692-1702

66. Macedo E, Malhotra R, Claure-Del GR, Fedullo P, Mehta RL. Defining urine output criterion for acute kidney injury in critically ill patients. *Nephrol Dial Transplant* 2011; 26: 509-515

67. Macedo E, Malhotra R, Bouchard J, Wynn SK, Mehta RL. Oliguria is an early predictor of higher mortality in critically ill patients. *Kidney Int* 2011; 80: 760-767

68. Endre ZH, Pickering JW, Walker RJ. Clearance and beyond: the complementary roles of GFR measurement and injury biomarkers in acute kidney injury (AKI). *Am J Physiol Renal Physiol* 2011; 301: F697-F707

69. Vanmassenhove J, Glorieux G, Hoste E, Dhondt A, Vanholder R, Van BW. Urinary output and fractional excretion of sodium and urea as indicators of transient versus intrinsic acute kidney injury during early sepsis. *Crit Care* 2013; 17: 1-10

70. Prowle JR, Liu YL, Licari E *et al*. Oliguria as predictive biomarker of acute kidney injury in critically ill patients. *Crit Care* 2011; 15: 1-10

71. Leedahl DD, Frazee EN, Schramm GE *et al*. Derivation of Urine Output Thresholds That Identify a Very High Risk of AKI in Patients with Septic Shock. *Clin J Am Soc Nephrol* 2014; 9:1-7

72. Cruz DN, Bolgan I, Perazella MA *et al*. North East Italian Prospective Hospital Renal Outcome Survey on Acute Kidney Injury (NEiPHROS-AKI): targeting the problem with the RIFLE Criteria. *Clin J Am Soc Nephrol* 2007; 2: 418-425

73. Haase M, Bellomo R, Matalanis G, Calzavacca P, Dragun D, Haase-Fielitz A. A comparison of the RIFLE and Acute Kidney Injury Network classifications for cardiac surgery-associated

acute kidney injury: a prospective cohort study. *J Thorac Cardiovasc Surg* 2009; 138: 1370-1376

74. Han SS, Kang KJ, Kwon SJ *et al*. Additional role of urine output criterion in defining acute kidney injury. *Nephrol Dial Transplant* 2012; 27: 161-165

75. Uchino S, Bellomo R, Goldsmith D, Bates S, Ronco C. An assessment of the RIFLE criteria for acute renal failure in hospitalized patients. *Crit Care Med* 2006; 34: 1913-1917

76. Anderson RJ, Linas SL, Berns AS *et al*. Nonoliguric acute renal failure. *New England Journal of Medicine* 1977; 296: 1134-1138

77. Pickering JW, Ralib AM, Endre ZH. Combining creatinine and volume kinetics identifies missed cases of acute kidney injury following cardiac arrest. *Crit Care* 2013; 17: 1-18

78. Poukkanen M, Vaara ST, Pettila V *et al*. Acute kidney injury in patients with severe sepsis in Finnish Intensive Care Units. *Acta Anaesthesiol Scand* 2013; 57: 863-872

79. Brown JR, Kramer RS, Coca SG, Parikh CR. Duration of acute kidney injury impacts long-term survival after cardiac surgery. *Ann Thorac Surg* 2010; 90: 1142-1148

80. Gottlieb SS, Abraham W, Butler J *et al*. The prognostic importance of different definitions of worsening renal function in congestive heart failure. *J Card Fail* 2002; 8: 136-141

81. Lassnigg A, Schmid ER, Hiesmayr M *et al*. Impact of minimal increases in serum creatinine on outcome in patients after cardiothoracic surgery: do we have to revise current definitions of acute renal failure? *Crit Care Med* 2008; 36: 1129-1137

82. Newsome BB, Warnock DG, McClellan WM *et al*. Long-term risk of mortality and end-stage renal disease among the elderly after small increases in serum creatinine level during hospitalization for acute myocardial infarction. *Arch Intern Med* 2008; 168: 609-616

83. Sasse KC, Nauenberg E, Long A, Anton B, Tucker HJ, Hu TW. Long-term survival after intensive care unit admission with sepsis. *Critical Care Medicine* 1995; 23: 1040-1047

84. Ponte B, Felipe C, Muriel A, Tenorio MT, Liano F. Long-term functional evolution after an acute kidney injury: a 10-year study. *Nephrology Dialysis Transplantation* 2008; 23: 3859-3866

85. Pereira MB, Zanetta DM, Abdulkader RC. The real importance of pre-existing comorbidities on long-term mortality after acute kidney injury. *PLoS ONE 2012;7(10):1-12*

86. Linder A, Fjell C, Levin A, Walley KR, Russell JA, Boyd JH. Small acute increases in serum creatinine are associated with decreased long-term survival in the critically ill. *Am J Respir Crit Care Med* 2014; 189: 1075-1081

87. Waikar SS, Bonventre JV. Creatinine kinetics and the definition of acute kidney injury. *J Am Soc Nephrol* 2009; 20: 672-679

88. Lisowska-Myjak B. Serum and urinary biomarkers of acute kidney injury. *Blood Purif* 2010; 29: 357-365

89. Coca SG, Yalavarthy R, Concato J, Parikh CR. Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney Int* 2008; 73: 1008-1016

90. Lameire NH, Vanholder RC, Van Biesen WA. How to use biomarkers efficiently in acute kidney injury. *Kidney Int* 2011; 79: 1047-1050

91. Bolignano D, Lacquaniti A, Coppolino G *et al*. Neutrophil gelatinase-associated lipocalin as an early biomarker of nephropathy in diabetic patients. *Kidney Blood Press Res* 2009; 32: 91-98

92. Brunner HI, Mueller M, Rutherford C *et al*. Urinary neutrophil gelatinase-associated lipocalin as a biomarker of nephritis in childhood-onset systemic lupus erythematosus. *Arthritis Rheum* 2006; 54: 2577-2584

93. Parravicini E, Nemerofsky SL, Michelson KA *et al*. Urinary neutrophil gelatinase-associated lipocalin is a promising biomarker for late onset culture-positive sepsis in very low birth weight infants. *Pediatr Res* 2010; 67: 636-640

94. Trachtman H, Christen E, Cnaan A *et al*. Urinary neutrophil gelatinase-associated lipocalcin in D+HUS: a novel marker of renal injury. *Pediatr Nephrol* 2006; 21: 989-994

95. Bolignano D, Lacquaniti A, Coppolino G, Campo S, Arena A, Buemi M. Neutrophil gelatinase-associated lipocalin reflects the severity of renal impairment in subjects affected by chronic kidney disease. *Kidney Blood Press Res* 2008; 31: 255-258

96. Mitsnefes MM, Kathman TS, Mishra J *et al*. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in children with chronic kidney disease. *Pediatr Nephrol* 2007; 22: 101-108

97. Murray PT, Mehta RL, Shaw A *et al*. Potential use of biomarkers in acute kidney injury: report and summary of recommendations from the 10th Acute Dialysis Quality Initiative consensus conference. *Kidney Int* 2014; 85: 513-521

98. Metzger J, Kirsch T, Schiffer E *et al*. Urinary excretion of twenty peptides forms an early and accurate diagnostic pattern of acute kidney injury. *Kidney Int* 2010; 78: 1252-1262

99. Kashani K, Al-Khafaji A, Ardiles T *et al*. Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. *Crit Care* 2013; 17: 1-12

100. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 2009; 54: 1012-1024

101. Haase M, Devarajan P, Haase-Fielitz A *et al*. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. *J Am Coll Cardiol* 2011; 57: 1752-1761

102. Mori K, Lee HT, Rapoport D *et al*. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. *J Clin Invest* 2005; 115: 610-621

103. Schmidt-Ott KM, Mori K, Li JY *et al*. Dual action of neutrophil gelatinase-associated lipocalin. *J Am Soc Nephrol* 2007; 18: 407-413

104. Xu SY, Pauksen K, Venge P. Serum measurements of human neutrophil lipocalin (HNL) discriminate between acute bacterial and viral infections. *Scand J Clin Lab Invest* 1995; 55: 125-131

105. Soni SS, Cruz D, Bobek I *et al*. NGAL: a biomarker of acute kidney injury and other systemic conditions. *Int Urol Nephrol* 2010; 42: 141-150

106. Decavele AS, Dhondt L, De Buyzere ML, Delanghe JR. Increased urinary neutrophil gelatinase associated lipocalin in urinary tract infections and leukocyturia. *Clin Chem Lab Med* 2011; 49: 999-1003

107. Cavalier E, Bekaert AC, Carlisi A, Legrand D, Krzesinski JM, Delanaye P. Neutrophil gelatinase-associated lipocalin (NGAL) determined in urine with the Abbott Architect or in plasma with the Biosite Triage? The laboratory's point of view. *Clin Chem Lab Med* 2011; 49: 339-341

108. Clerico A, Galli C, Fortunato A, Ronco C. Neutrophil gelatinase-associated lipocalin (NGAL) as biomarker of acute kidney injury: a review of the laboratory characteristics and clinical evidences. *Clin Chem Lab Med* 2012; 50: 1505-1517

109. Grenier FC, Ali S, Syed H *et al*. Evaluation of the ARCHITECT urine NGAL assay: assay performance, specimen handling requirements and biological variability. *Clin Biochem* 2010; 43: 615-620

110. Haase-Fielitz A, Haase M, Devarajan P. Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury: a critical evaluation of current status. *Ann Clin Biochem* 2014; 51: 335-351

111. Lippi G, Aloe R, Storelli A, Cervellin G, Trenti T. Evaluation of NGAL Test, a fully-automated neutrophil gelatinase-associated lipocalin (NGAL) immunoassay on Beckman Coulter AU 5822. *Clin Chem Lab Med* 2012; 50: 1581-1584

112. Cai L, Rubin J, Han W, Venge P, Xu S. The origin of multiple molecular forms in urine of HNL/NGAL. *Clin J Am Soc Nephrol* 2010; 5: 2229-2235

113. Martensson J, Xu S, Bell M, Martling CR, Venge P. Immunoassays distinguishing between HNL/NGAL released in urine from kidney epithelial cells and neutrophils. *Clin Chim Acta* 2012; 413: 1661-1667

114. Jang HR, Rabb H. Immune cells in experimental acute kidney injury. *Nat Rev Nephrol* 2014; oct 21

115. Lippi G, Plebani M. Neutrophil gelatinase-associated lipocalin (NGAL): the laboratory perspective. *Clin Chem Lab Med* 2012; 50: 1483-1487

116. Martensson J, Bell M, Oldner A, Xu S, Venge P, Martling CR. Neutrophil gelatinase-associated lipocalin in adult septic patients with and without acute kidney injury. *Intensive Care Med* 2010; 36: 1333-1340

117. Bagshaw SM, Bennett M, Haase M *et al*. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. *Intensive Care Med* 2010; 36: 452-461

118. Dellinger RP, Levy MM, Rhodes A *et al*. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med* 2013; 41: 580-637

119. Di G, May CN, Bellomo R. Vital organ blood flow during hyperdynamic sepsis. *Chest* 2003; 124: 1053-1059

120. Gordon AC, Russell JA, Walley KR *et al*. The effects of vasopressin on acute kidney injury in septic shock. *Intensive Care Med* 2010; 36: 83-91

121. Rivers E, Nguyen B, Havstad S *et al*. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* 2001; 345: 1368-1377

122. Wan L, Bellomo R, May CN. The effect of normal saline resuscitation on vital organ blood flow in septic sheep. *Intensive Care Medicine* 2006; 32: 1238-1242

123. Doi K, Katagiri D, Negishi K *et al*. Mild elevation of urinary biomarkers in prerenal acute kidney injury. *Kidney Int* 2012; 82: 1114-1120

124. Nejat M, Pickering JW, Devarajan P *et al*. Some biomarkers of acute kidney injury are increased in pre-renal acute injury. *Kidney Int* 2012; 81: 1254-1262

125. Bellomo R, Bagshaw S, Langenberg C, Ronco C. Pre-renal azotemia: a flawed paradigm in critically ill septic patients? *Contrib Nephrol* 2007; 156: 1-9

126. Haase M, Kellum JA, Ronco C. Subclinical AKI--an emerging syndrome with important consequences. *Nat Rev Nephrol* 2012; 8: 735-739

127. Ronco C, Kellum JA, Haase M. Subclinical AKI is still AKI. *Crit Care* 2012; 16(3): 1-4

128. Palevsky PM, Zhang JH, O'Connor TZ *et al*. Intensity of renal support in critically ill patients with acute kidney injury. *N Engl J Med* 2008; 359: 7-20

129. Choi JS, Kim YA, Kim MJ *et al*. Relation between transient or persistent acute kidney injury and long-term mortality in patients with myocardial infarction. *Am J Cardiol* 2013; 112: 41-45

130. Uchino S, Bellomo R, Bagshaw SM, Goldsmith D. Transient azotaemia is associated with a high risk of death in hospitalized patients. *Nephrol Dial Transplant* 2010; 25: 1833-1839

131. Goldberg A, Kogan E, Hammerman H, Markiewicz W, Aronson D. The impact of transient and persistent acute kidney injury on long-term outcomes after acute myocardial infarction. *Kidney Int* 2009; 76: 900-906

132. Bagshaw SM, Haase M, Haase-Fielitz A, Bennett M, Devarajan P, Bellomo R. A prospective evaluation of urine microscopy in septic and non-septic acute kidney injury. *Nephrol Dial Transplant* 2012; 27: 582-588

133. Darmon M, Vincent F, Dellamonica J *et al*. Diagnostic performance of fractional excretion of urea in the evaluation of critically ill patients with acute kidney injury: a multicenter cohort study. *Crit Care* 2011; 15: 1-8

134. Espinel CH. The FENa test. Use in the differential diagnosis of acute renal failure. *JAMA* 1976; 236: 579-581

135. Gotfried J, Wiesen J, Raina R, Nally JV, Jr. Finding the cause of acute kidney injury: which index of fractional excretion is better? *Cleve Clin J Med* 2012; 79: 121-126

136. Nanji AJ. Increased fractional excretion of sodium in prerenal azotemia: need for careful interpretation. *Clinical Chemistry* 1981; 27: 1314-1315

137. Steiner RW. Interpreting the fractional excretion of sodium. *American Journal of Medicine* 1984; 77: 699-702

138. Zarich S, Fang LS, Diamond JR. Fractional excretion of sodium. Exceptions to its diagnostic value. *Archives of Internal Medicine* 1985; 145: 108-112

139. Vaz AJ. Low fractional excretion of urine sodium in acute renal failure due to sepsis. *Arch Intern Med* 1983; 143: 738-739

140. Dal C, Fuiano G, Conte G *et al*. Mechanism of increased plasma urea after diuretic therapy in uraemic patients. *Clinical Science* 1985; 68: 255-261

141. Kaplan AA, Kohn OF. Fractional excretion of urea as a guide to renal dysfunction. *American Journal of Nephrology* 1992; 12: 49-54

142. Thijs A, Thijs LG. Pathogenesis of renal failure in sepsis. *Kidney International Suppl* 1998; 66: S34-S37

143. Goldstein MH, Lenz PR, Levitt MF. Effect of urine flow rate on urea reabsorption in man: urea as a "tubular marker". *Journal of Applied Physiology* 1969; 26: 594-599

144. Carvounis CP, Nisar S, Guro-Razuman S. Significance of the fractional excretion of urea in the differential diagnosis of acute renal failure. *Kidney International* 2002; 62: 2223-2229

145. Pepin MN, Bouchard J, Legault L, Ethier J. Diagnostic performance of fractional excretion of urea and fractional excretion of sodium in the evaluations of patients with acute kidney injury with or without diuretic treatment. *Am J Kidney Dis* 2007; 50: 566-573

146. Bagshaw SM, Langenberg C, Bellomo R. Urinary biochemistry and microscopy in septic acute renal failure: a systematic review. *Am J Kidney Dis* 2006; 48: 695-705

147. Bagshaw SM, Gibney RT. Acute kidney injury: clinical value of urine microscopy in acute kidney injury. *Nat Rev Nephrol* 2009; 5: 185-186

148. Schrier RW. Urinary indices and microscopy in sepsis-related acute renal failure. *American Journal of Kidney Diseases* 2006; 48: 838-841

149. Dewitte A, Biais M, Petit L *et al*. Fractional excretion of urea as a diagnostic index in acute kidney injury in intensive care patients. *J Crit Care* 2012; 27: 505-510

CHAPTER 8:

CONCLUSIONS

8.1 Definition of AKI

Despite the fact that several associations and guideline bodies established criteria to define AKI, epidemiological data on AKI continue to vary widely, even within the same setting. This is predominantly explained by dissimilarity in using the RIFLE, AKIN, KDIGO and ERBP criteria. Although these criteria are originally based on both the sCr and the urinary output criterion, the latter is often omitted.

We used different criteria to define AKI, either including vs not including the urinary output criterion, using absolute vs relative sCr changes and changing the baseline value (either a historical baseline, an estimated baseline value or the ICU admission value). By doing this we found a widely different prevalence of AKI ranging from 14% to 79% depending on which definition was used. Overall, prevalence decreased from 45-79% till 14%-54% if the urinary output was not included, highlighting the importance of using both criteria when defining AKI.

8.2 AKI and mortality

In our sepsis cohort, overall mortality was high, even after ICU survival. We only found an association between AKI and mortality if AKI was defined as an absolute sCr increase between the value 24 hours after ICU admission and the ICU admission value. However, when AKI was defined based on a highest value over a certain span or when referring to a historical or an estimated baseline value, no such association was observed. This underlines the importance of including a sCr increase that reflects a potential response to specific therapy such as fluid resuscitation instead of using the highest sCr value over a certain time span without taking into account the evolution of sCr. Also, the ICU admission value should be used as the baseline value.

As in the cardiac surgery setting, the lowest still robust cut-off for sCr increase in sepsis is 0.3mg/dl. However, although a 0.3mg/dl increase of sCr was associated with mortality in univariate analysis, this was no longer the case after adjusting for severity of illness. This indicates that outcome in septic AKI is mainly determined by severity of illness and factors that were already present before ICU admission, rather than by AKI itself.

8.3 Biomarkers for diagnosis of AKI

The difficulties in early diagnosis and the largely unknown pathophysiology of septic AKI, partly explain why the successful results in animal studies have not been translated to humans. Although the quest for better biomarkers and their allegedly promising results might be fascinating from the scientific point of view, their use in daily clinical practice currently does not outperform standard markers such as clinical models including sCr and urinary output.

In a systematic review we evaluated the performance of new biomarkers to diagnose AKI and found that performance was variable and inconsistent. Overall, biomarkers performed better when the exact timing of the renal event was known (e.g. in cardiac surgery) and in children without comorbidities.

Especially in septic AKI the use of biomarkers becomes complex. First, there is no clear timing of the event and second, several biomarkers, such as NGAL and IL-18, can be influenced by inflammation, irrespective of AKI.

It seems highly unlikely that one single biomarker can cover the entire spectrum of AKI. However, the use of a panel of biomarkers is expensive, time-consuming and not readily available.

The use of biomarkers for diagnosis of AKI in daily clinical practice is currently not justified and a clinical model including sCr and urinary output remains the gold standard.

8.4 Transient vs Intrinsic AKI

The discrimination between transient and intrinsic AKI and thus between those who would potentially benefit from further fluid resuscitation vs those who could rather experience harm from it, remains an important issue in clinical nephrology.

We found a gradual increase in urinary NGAL in patients with no-AKI vs transient AKI vs intrinsic AKI, but only if AKI was diagnosed according to the sCr criterion. If based on the urinary output criterion, there was no significant difference in NGAL levels between no-AKI and transient AKI, demonstrating that recording urinary output might allow for AKI diagnosis (and thus treatment) at a stage when there is no tubular damage yet.

The performance of urinary indices such as FENa and FEUrea to differentiate between transient and intrinsic AKI has been questioned. We found that neither FENa, FEUrea or NGAL alone, showed a good discriminative value. However, a combination of FENa and FEUrea was helpful in differentiating transient and intrinsic AKI. Whereas a high FENa and FEUrea is strongly predictive of transient AKI, a high FENa and a low FEUrea is predictive of intrinsic AKI. The high prevalence of a very low FENa in early sepsis, questions the value of the historically applied 1% cut-off value to differentiate between transient and intrinsic AKI and suggests that it should be lower.

8.5 Correlation between sNGAL and uNGAL

In sepsis, the main determinant of urinary NGAL is serum NGAL, attributed to overflow from the systemic circulation and/or reduced tubular reabsorption, irrespective of presence of AKI.

Both serum and urinary NGAL levels are influenced by severity of illness and inflammation, as assessed by APACHE II and CRP.

Although uNGAL levels were significantly different, but not discriminative, between no-AKI, transient and intrinsic AKI, this was no longer the case when classified according to severity of illness (sepsis patients without vs with shock).

These findings caution for the use of NGAL for AKI diagnosis in sepsis.

8.6 Pathophysiology of septic AKI

We found indirect evidence for a role of renal hypoperfusion in early sepsis by demonstrating that the majority of patients had a FENa<1% at ICU admission. The latter indirectly points to an adequate response of the kidney to a state of decreased glomerular perfusion pressure with maximal salt and water retention. This was also corroborated by the fact that we found an increase in AKI prevalence, with decreasing quartiles of FENa if AKI was defined according to the urinary output criterion, suggesting that as patients are retaining more salt and water, they become more oliguric.

We also found that some patients classified as having transient AKI had no signs of tubular injury (as assessed by FENa and NGAL) at admission and still did not have signs of tubular injury 24 hours later. Some patients classified as having intrinsic AKI did not have signs of tubular injury at admission but did so 24 hours later. These findings are compatible with the existence of a continuum between transient AKI without tubular injury, transient AKI with minor tubular injury and intrinsic AKI, and underline the role of renal hypoperfusion in the pathophysiology of early sepsis.

8.7 Future perspectives

The importance of uniformity in defining AKI should continue to be stressed. The effect of incorporating the evolution of serum creatinine in the AKI definition and of using the ICU admission value as a baseline value needs to be validated in larger prospective studies, in different settings.

There is no discussion on the shortcomings of the currently used markers, such as serum creatinine, FENa, FEUrea and urinary output for diagnosing AKI. This implies that the search for a new troponin-like biomarker with high sensitivity and specificity concerning tubular damage, should remain an important target. However, it is unlikely that one biomarker will cover the entire spectrum of AKI and different settings such as sepsis, cardiac surgery etc, should be individually explored for potential new biomarkers. More emphasis should also be put on the analytical issues regarding the method that is being used to quantify the biomarker(s) and on standardization of sample collection and preparation, biomarker normalization for age, gender and/or urine flow rate and determination of cut-off values to distinguish between the diseased and the non-diseased state.

Long term outcome data in exclusively sepsis patients are scarce. There is an ongoing need to investigate long term consequences of septic AKI with regard to increased risk for mortality and development of CKD after AKI compared to no-AKI. Although it is generally well accepted that AKI is an independent predictor for short and long term mortality and increased risk for CKD development, there is still controversy on whether this also stands in sepsis, which can be considered as a different clinical entity. Also the association between

duration of AKI and outcome should be further explored since several reports indicate that it is mainly the duration of AKI that is associated with mortality.

ACKNOWLEDGEMENTS

Acknowledgements

I would like to thank Prof Dr. Raymond Vanholder for the opportunities given over the years. I would also like to thank Prof Dr. Annemieke Dhondt and Prof Dr. Eric Hoste for supporting this thesis. A special thank you to Prof Dr. Norbert Lameire for his valuable input, and to the reading and examining committee for their critical reviews which truly helped improving this work.

I can genuinely say that over the years, I have felt surrounded by many great and warm people, family and friends, who each in their own way helped me during this sometimes difficult process. You will hopefully forgive me for not naming all of you personally.

I am heartily thankful to my supervisor, Prof Dr. Wim Van Biesen. Although he compared my haircut with the fur of a rabbit and several other mammals, repeatedly promised me one-way tickets to far African or Eastern European countries and accused me of shortening his life span on several occasions, I still managed to like him. Wim, thank you so much for guiding me through this forest of data, for having great ideas, for your down-to-earth approach and especially thank you for being my friend. I hope we'll continue to annoy each other over the next few decades.

An enormous thank you to Dirk, my gorgeous husband, my yummy-cook, my David-Bowie-fan, my lover-and-my-best-friend, for bringing some art into my scientific world, for making me laugh every day, even when I'm feeling sad, but especially for spoiling me with all the love I could ever have wished for.

Marion! My soulmate, my-almost-sister, my sweetheart. Thank you so much for being in my life. I truly wish you are going to marry a Flemish guy and buy a house in our street.

Annick, my friend and best-nurse-in-the-universe, thank you for always just being a phone call away.

A wonderful thank you to my colleagues and friends Steven and Evi who make daily life more pleasant. Evi, I would like to take the opportunity to ask you to stop your despicable project of growing fungi in our office.

Thanks to all secretaries and nurses in Gent and Geraardsbergen, to all of my colleagues and to the people from the lab. A warm thank you to Christel and Benoit for spending hours helping me and never losing their smile.

Jill

CURRICULUM VITAE

Curriculum vitae Vanmassenhove J, MD (°30/03/1978)

Consultant, Department of Nephrology, Ghent University Hospital

- Legal degree of Doctor in Medicine, Surgery and Obstetrics (MD) at the Ghent University (Rijksuniversiteit Gent) in 2003 with great distinction.
- Legal degree of Specialist in Internal Medicine in 2008 (Ghent University)
- Legal degree of Specialist in Nephrology in 2009 (Ghent University)
- Resident staff member of Nephrology Dept Ghent University since 2009
- Accreditated doctor in the LOK 0247 of Nephrology

Courses

- 2007: Harvard course on Nephrology, Boston, USA
- 01-02/2011: Course in Statistics organised by the Institute for Continuing Education in Science of the Ghent University

Oral presentations on national or international meetings

- 01-12-2011: NBVN meeting: New urinary and serum biomarkers for diagnosing of AKI: too little too early.
- 08-12-2011: staff meeting internal medicine Ghent University Hospital: Nieuwe diagnostische technieken. De zin en onzin van nieuwe biomarkers voor de vroegtijdige diagnose van AKI.
- Oral communication at ERA-EDTA 2011: Renal hypoperfusion, rather than tubular damage is the main initial event in AKI during early sepsis.
- 27-03-2013: 6de Belgische Dialyse Symposium 26-28 maart 2013, Hargimont, België: Zin en onzin van nieuwe biomarkers in de diagnostiek van AKI.
- 21-05-2013: 50th ERA-EDTA Congress May 18-21 2013, Instanbul, Turkey. Sepsis in the ICU: cause or consequence of AKI.
- 26-10-2013: The 8th National Congress of Nephrology. October 24-26 2013, Sibiu, Romania. The kidney in sepsis. Biomarkers in AKI diagnosis.
- 21-11-2013: MANAMA (education for trainees): electrolyte and acid-base disorders

Publications:

- J. Vanmassenhove, V. Van Maele en H. Fransen. Case Report: een 50-jarige man met hevige anabole pijn en braken. Tijdschrift voor Geneeskunde, 2008 (64), 17:872-875
- Vanmassenhove J, Vanholder R, Forsyth R, Dhondt A. Encapsulating peritoneal sclerosis in a patient with primary hyperoxaluria type 1. Perit Dial Int. 2010 Jan-Feb; 30(1):108-111.
- Vanmassenhove J, Sallée M, Guilpain P, Vanholder R, De Potter A, Libbrecht L, Suarez F, Hermine O, Fakhouri F. Fanconi syndrome in lymphoma patients: report of the first case series. Nephrol Dial Transplant. 2010 Aug; 25(8): 2516-2520.
- Vanholder R, Borniche D, Claus S, Correa-Rotter R, Crestani R, Ferir MC, Gibney N, Hurtado A, Luyckx VA, Portilla D, Rodriguez S, Sever MS, Vanmassenhove J, Wainstein R. When the earth trembles in het Americas: The experience of Haiti and Chile 2010. Nephron Clin Pract. 2010 Aug 30; 117(3):c184-c197.

- Ghoul BE, Squalli T, Servais A, Elie C, Meas-Yedid V, Trivint C, Vanmassenhove J, Grünfeld JP, Olivo-Marin JC, Thervet E, Noël LH, Prié D, Fakhouri F. Urinary procollagen III aminoterminal propeptide (PIIINP): a fibrotest for the nephrologist. Clin J Am Soc Nephrol. 2010 Feb; 5(2): 205-210.
- Touzot M, Elie C, Vanmassenhove J, Maillard N, Buzyn A, Fakhouri F. Long-term renal function after a haematopoietic stem cell transplantation: a single-centre study. Nephrol Dial Transplant. 2010 Feb; 25(2): 624-7.
- Van Biesen W, Vanmassenhove J, Hoste E, Vanholder R. Defining AKI: playing hide-and-seek with the unknown man. NDT 2011 Feb 26(2) 399-401.
- Van Biesen W, Vanmassenhove J, Van Canneyt K, Vanholder R, Eloot S. Influence of switching connection ports of double-lumen permanent tunnelled catheters on total solute removal during dialysis. J Nephrol. 2011 May-Jun; 24 (3): 338-44.
- Van Biesen W, Vanmassenhove J, Lameire N, Vanholder R. Does urinary neutrophil gelatinase-associated lipocalin really solve the issue of discriminating prerenal from intrinsic acute kidney injury. Kidney Int. 2012 Feb; 8(13): 321.
- Maddens B, Ghesquiere B, Vanholder R, Demon D, Vanmassenhove J, Gevaert K, Meyer E. Chitinase-like proteins are candidate biomarkers for sepsis-induced AKI. Mol Cell Proteomics. 2012 Jan 10
- Lameire N, Vanmassenhove J, Van Biesen W. What is the difference between prerenal and renal acute kidney injury. Acta Clinica Belgica. 2012 sept-oct; 67(5):309-314
- Vanmassenhove J, Vanholder R, Nagler E, Van Biesen W. Urinary and serum biomarkers for the diagnosis of acute kidney injury: an in-depth review of the literature. NDT 2013 Feb 28(2):254-273
- Vanmassenhove J, Glorieux G, Hoste E, Dhondt A, Van Biesen W. Urinary output and fractional excretion of sodium and urea as indicators of transient versus intrinsic acute kidney injury during early sepsis. Crit Care 2013, 17(5):R234.
- Vanmassenhove J, Glorieux G, Hoste E, Dhondt A, Vanholder R, Van Biesen W. AKI in early sepsis is a continuum from transient AKI without tubular damage over transient AKI with minor tubular damage to intrinsic AKI with severe tubular damage. Int Urol Nephrol. 2014 Sep 5.

Abstracts/Posters

- Jill Vanmassenhove, Raymond Vanholder, Griet Glorieux, Wim Van Biesen. Diagnostic impact of diuresis and creatinine in RIFLE: poster presentation at the ERA-EDTA Congress, Prague, June 23-26 2011
- Jill Vanmassenhove, Raymond Vanholder, Griet Glorieux, Wim Van Biesen. First day evolution of serum creatinine and not NGAL predicts mortality in ICU patients with sepsis: poster presentation at the ERA-EDTA Congress, Prague, June 23-26 2011
- Jill Vanmassenhove, Raymond Vanholder, Griet Glorieux, Wim Van Biesen. Neural networks for the diagnosis of Acute Kidney Injury: poster presentation at the ERA-EDTA Congress, Paris, May 24-27 2012
- Jill Vanmassenhove, Raymond Vanholder, Griet Glorieux, Wim Van Biesen. Urinary NGAL at admission does not provide additional information than serum creatinine, urine output and first 24h fluid balance, for prediction of AKI in septic patients at ICU: poster presentation at the ERA-EDTA Congress, Paris, May 24-27 2012

- Jill Vanmassenhove, Raymond Vanholder, Griet Glorieux, Wim Van Biesen. Renal hypoperfusion, rather than tubular damage is the main initial event in AKI during early sepsis: poster presentation at the ERA-EDTA Congress, Paris, May 24-27 2012
- Jill Vanmassenhove, Eric Hoste, Griet Glorieux, Annemieke Dhondt, Raymond Vanholder, Wim Van Biesen. Assessment of different cut-off levels of serum creatinine increase to predict mortality in septic patients: poster presentation at the ERA-EDTA Congress, Istanbul, May 18-21 2013.
- Jill Vanmassenhove, Eric Hoste, Griet Glorieux, Annemieke Dhondt, Raymond Vanholder, Wim Van Biesen. Predictors associated with short and median-term outcome of patients admitted to ICU with sepsis: poster presentation at the ERA-EDTA Congress, Istanbul, May 18-21 2013.
- Jill Vanmassenhove, Eric Hoste, Griet Glorieux, Annemieke Dhondt, Raymond Vanholder, Wim Van Biesen. Median term outcome of renal function in patients admitted to ICU with sepsis: poster presentation at the ERA-EDTA Congress, Istanbul, May 18-21 2013.
- Evi Nagler, Jill Vanmassenhove, Sabine van der Veer, Ionut Nistor, Wim Van Biesen, Angela Webster, Raymond Vanholder. Diagnosis and treatment of hyponatraemia: a systematic review of clinical practice quidelines: poster presentation at the ERA-EDTA Congress, Istanbul, May 18-21 2013.
- Fraselle S, De Cremer K, Vanmassenhove J, Glorieux G, Bolle F, Van Overmeire L, Van Loco J, Van Biesen W, Vanholder R. A targeted proteomics method to detect urinary biomarkers of kidney disease: poster presentation 29 and 30/11/2012 Ghent BePA (Belgian Proteomics Associations) conference
- Fraselle S, De Cremer K, Coucke W, Glorieux G, Vanmassenhove J, Schepers E, Neirynck N, Van Overmeire I, Van Loco J, Van Biesen W, Vanholder R. A protein biomarker panel to discriminate progressive from non-progressive chronic kidney disease using a targeted proteomics method. HUPO 5-8/10/2014 Madrid

Foreign Medical experience

- Mai - July 2002: Kaunas, Lithuania: Obstetrics and Surgery.
- Oct - Dec 2002: Cape Town, South Africa: Family Medicine, Internal Medicine and Obstetrics.
- 2007 - 2008: Hôpital Necker, Service de Néphrologie/maladies métaboliques, Paris, France. Scolarship via ‘le Collège de médécine des hôpitaux de Paris’.
- 2011: Haïti Earthquake as a member of RDRTF

Membership:

- RDRTF (Renal Disaster Relief Task Force)

Languages

- Mother tongue: Dutch
- French, English, German, Italian, Spanish: excellent